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Comparison of the Sodium Dependency of Uptake of *meta*-Iodobenzylguanidine and Norepinephrine into Cultured Bovine Adrenomedullary Cells

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SUMMARY

Radioiodinated *meta*-iodobenzylguanidine (MIBG), a scintigraphic agent used for the detection of human pheochromocytomas, is thought to utilize the same uptake and retention mechanism(s) as norepinephrine (NE). Using cultured bovine adrenomedullary cells, we compared the mechanism(s) of uptake of MIBG to that of NE. Two different uptake systems were identified. NE and MIBG were taken up by a sodium-dependent system that was characterized by: 1) temperature dependency; 2) high affinity: K_m of 1.22 ± 0.12 μM for MIBG and 1.41 ± 0.50 μM for NE; 3) low capacity: V_m (picomoles/10^6 cells/10 min) of 64.3 ± 3.3 for MIBG and 36.6 ± 7.2 for NE; 4) saturability; 5) ouabain sensitivity; and 6) energy dependency. However, NE and MIBG also were taken up by a temperature-dependent, sodium-independent, apparently unsaturable, and energy-independent system. The sodium-dependent uptake system fulfills many of the criteria for $Uptake_1$ whereas the sodium-independent uptake system is most likely a passive diffusion process. NE uptake proceeded predominantly by the sodium-dependent process. Uptake of MIBG occurred by both pathways at low concentrations, but at high concentrations (>10 μM) uptake was predominantly (75 to 100%) by the sodium-independent process. Inhibition studies suggest that MIBG and NE are transported by the same carrier involved in the sodium-dependent system. Scintiscans of the human adrenals and pheochromocytomas appear to reflect uptake of [^{131}I]MIBG by the sodium-dependent system.

INTRODUCTION

Since we initially reported the development of radioiodinated MIBG[1] as a potential adrenal imaging agent (1, 2), we have demonstrated that [^{131}I]MIBG scintigraphy is an important and safe clinical technique for the visualization of benign and malignant pheochromocytomas and adrenomedullary hyperplasias (3–7). This agent currently is being evaluated in the treatment of human pheochromocytomas (8, 9). Although the use of [^{131}I]MIBG is becoming worldwide (10–12), the mechanism(s) of its uptake and concentration into adrenomedullary tissue is not known. Preliminary studies have suggested that the uptake and retention mechanism(s) for MIBG parallel those for NE (2, 13).

To better understand the pharmacology underlying the scintigraphic images of pheochromocytomas, we have used cultured bovine adrenomedullary cells as a model system to assess rigorously the similarities and dissimilarities of NE and MIBG uptake. Although these cells have been cultured in numerous laboratories, only a single report, to date, was concerned with the mechanism(s) of NE uptake (14). These investigators concluded that NE uptake into adrenomedullary cells was via a high affinity uptake system; however, these studies did not rigorously assess the sodium dependency and energy requirements of this uptake. We now report the identification of two uptake systems which appear to operate independently and overlap at high and low concentrations: 1) a sodium-dependent system which fulfills many of the criteria for $Uptake_1$, and 2) a sodium-independent system which, most likely, is a simple diffusion process.

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[1] The abbreviations used are: MIBG, *meta*-iodobenzylguanidine; NE, norepinephrine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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MATERIALS AND METHODS

Chemicals, radiochemicals, and materials. Collagenase (type I, 130 to 250 units/mg; type V, 300 to 500 units/mg), DNase (200 to 300 Kuntz units/mg, trypsin (9 to 12,000 *N*-α-benzoyl-L-arginine ethyl ester units/mg), *l*-norepinephrine, ouabain, ascorbic acid, bovine serum albumin, HEPES, cytosine arabinoside, 2-deoxy-D-glucose, and sodium azide were purchased from Sigma Chemical Co. (St. Louis, MO). Nonradiolabeled MIBG was synthesized by the method of Wieland *et al.* (1). Four-well Nunc Multidish plates were obtained from Vanguard International (Neptune, NJ). Minimum essential medium with Earle's salt solution, fetal calf serum, penicillin, and streptomycin were purchased from K. C. Biologicals (Lenexa, KS). Norepinephrine, *levo-ring*-2,5,6-^{3}H-labeled with a specific activity of 40 to 60 Ci/mmol was obtained from New England Nuclear (Boston, MA). Biocount scintillation fluid was purchased from Research Products International (Mt. Prospect, IL). Using an iodide exchange technique (15), [^{125}I]MIBG was prepared at a specific activity of 1.5 to 2.3 Ci/mmol. All other reagents were analytic grade or higher purity.

Preparation, maintenance, and characterization of cultured adrenomedullary cells. Bovine adrenals, obtained at a local slaughterhouse immediately after exsanguination, were freed of connective tissue and transported on ice in a Ca^{2+}, Mg^{2+}-free Locke's buffer to the laboratory. The adrenomedullary cells were dispersed using modifications of the technique of Kilpatrick *et. al.* (16). The modifications are: 1) the collagenase solution, approximately 0.05%, consisted of two parts of type V collagenase, adjusted to represent a constant activity of 300 units/mg, and one part of type I collagenase, adjusted to represent a constant activity of 200 units/mg; and 2) after the first *in vitro* digestion with collagenase, we added approximately 15% of a 0.25% trypsin solution to each subsequent collagenase digestion. Freshly dispersed cells were plated at densities of 0.6 to 0.8 × 10^6 cells per well in Nunc Multidish plates and maintained in minimum essential medium with Earle's salt solution supplemented with 15% fetal calf serum, penicillin (100 μg/ml), and streptomycin (100 μg/ml). Fibroblast growth was inhibited by the addition of 10 μM cytosine arabinoside. The cells were maintained at 37° in a humidified incubator (Heraeus) under a CO_2:air (5.95) atmosphere and allowed to adapt to culture for 4 days prior to starting an experiment. After this adaptation period, the medium was changed every second or third day. Twenty-four hr prior to commencing an experiment, the medium was replaced with fresh medium without cytosine arabinoside. We have found that uptake was maximal when cytosine arabinoside was removed 18 to 24 hr prior to the experiment.[2] Prior to plating the cells and/or commencing an experiment, cell viability was always 95% or greater by trypan blue exclusion. Cell catecholamine content was assayed routinely using the differential assay technique of Anton and Sayre (17), and total catecholamines were within the range of 30 to 40 nmol/10^6 cells. Histofluorescence was used periodically to further characterize the cell dispersions (18). All experiments were performed 4 to 8 days after dispersion.

Incubation conditions and procedures. During the uptake studies, the tissue culture plates were placed in a 37° water bath or an ice water bath (0.5 to 1.5°). Fifteen min prior to commencing the uptake study, the minimum esssential medium with Earle's salt solution culture media was removed, and the cells were preincubated, at either temperature, with 0.5 ml of HEPES-buffered Krebs-Ringer glucose (H-KRG buffer: 125 mM NaCl, 4.8 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5.6 mM glucose, and 25 mM HEPES), containing 1 mM ascorbic acid, adjusted to a final pH of 7.35. After the preincubation, the H-KRG solution was removed, and the cells in each well were incubated with 0.30 ml of H-KRG solution containing various concentrations of either [^{3}H]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 [^{3}H]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 [^{3}H]NE and [^{125}I]MIBG taken up by the cells was extracted by the addition of 0.30 ml of 10% trichloroactetic 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 Biocount scintillant, the vials containing the radioactivity due to [^{3}H]NE were mixed well and counted in a Beckman LS 7500 microprocessor-controlled liquid scintillation system. The samples were counted for 10 min or to the preset Sigma error of 0.50%. The tubes containing radioactivity due to [^{125}I]MIBG were mixed well and counted in a Packard model 5230 Autogamma scintillation spectrometer with an efficiency of 77.5%. The 37° incubations were done in quadruplicate, while the 0° incubations were done in duplicate. The total uptake at a given time point or concentration was determined by correcting the uptake at 37° for nonspecific uptake of 0°.

Uptake kinetics and sodium dependency of uptake. Kinetic studies were done by incubating the cells in the presence of 0.25 to 50 μM NE or MIBG to determine the K_m (in micromolar) and V_m (in picomoles/10^6 cells/10 min) for each agent. For determination of the sodium dependency of uptake, two kinetic studies for a single agent, either [^{3}H]NE or [^{125}I]MIBG, were performed on the same day. One kinetic study was done by incubating the cells in each well with the agent in 0.30 ml of H-KRG solution, while the second, complementary, kinetic study was done by incubating the cells with the same agent at identical concentrations in 0.30 ml of "zero-sodium" H-KRG solution. 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 mM).

Ouabain and metabolic inhibition of uptake. Cells were incubated in zero-sodium H-KRG containing 0.5 and 20 μM of either [^{3}H]NE or [^{125}I]MIBG in the presence or absence of ouabain. Specifically, stock solutions of 5 mM ouabain were prepared in the appropriate buffer and diluted so that the final concentration in the incubation medium was 1.0, 6.4, 10, 64, 100, or 1000 μM. In order to determine the time dependency of the ouabain inhibition of the sodium-dependent uptake, cells were preincubated with either 3.2 or 5.6 μM ouabain in H-KRG for various times at 37°, washed with H-KRG to remove the oubain, and incubated in H-KRG containing 0.5 μM of either [^{3}H]NE or [^{125}I]MIBG.

To assess the energy requirements for uptake, cells were preincubated at 37° for 30 min in zero-sodium H-KRG or H-KRG in the presence or absence of glucose with or without 5 mM 2-deoxy-D-glucose and/or 10 mM sodium azide. After washing the cells with the identical medium without a metabolic inhibitor, the cells were incubated in the identical medium containing 0.5 and 20 μM [^{3}H]NE or [^{125}I]MIBG to measure uptake. Some cells were preincubated and incubated in the presence of 5 mM 2-deoxy-D-glucose and 10 mM sodium azide.

Competitive inhibition of uptake. Cells were incubated in zero-sodium H-KRG or H-KRG containing 0.5 μM of [^{3}H]NE in the presence or absence of varying concentrations of unlabeled MIBG. Cells also were incubated in zero-sodium H-KRG or H-KRG containing 0.5 μM [^{125}I]MIBG in the presence or absence of varying concentrations of unlabeled NE.

Data analysis. All uptake studies, other than the time course of uptake, were conducted for 14 min, and the total uptake was expressed as picomoles/10^6 cells/10 min. Each experiment was replicated at least once and the results of a single, representative experiment are presented. Each data point represents the mean of quandruplicate values ± the standard deviation of the mean. The significance of difference between two points was evaluated using Student's *t*-test (19) and the significance of differences between multiple points was evaluated using a one-way analysis of variance in conjunction with Duncan's new multiple range test (19). The kinetic studies were analyzed using the double-reciprocal equation of Lineweaver-Burk and/or by the Eadie-Hoffstee equation, and a standard deviation for each parameter was calculated (20). The IC_{50} micromolar values were obtained from the linear portion of semilogarithmic plots of per cent inhibition versus

[2] S. Jaques, Jr. and M. C. Tobes, unpublished observations.

concentration. Regression lines were calculated by least squares analyses of all data points for both kinetic and inhibition studies.

RESULTS

Temperature dependency of uptake. As shown in Fig. 1, the uptake of 0.25 μM NE and 0.25 μM MIBG was temperature-dependent. The uptake of each agent at 37° was linear to at least 20 min, and always greater than that at 0° for all concentrations of NE and MIBG studied. Accumulation at 0° was a small and constant fraction of the total external concentration over time at all concentrations of NE and MIBG; therefore, uptake at 0° was characteristic of a nonspecific process. The total uptake by the temperature-dependent process was calculated as the uptake at 37° less that at 0°.

Kinetics of uptake. We evaluated the kinetics of both NE and MIBG to determine if the total uptake for either or both agents was a saturable process. As shown in Fig. 2A, the total uptake of NE appeared to be a saturable process, with an apparent K_m of 1.42 ± 0.15 μM and a V_m of 48.1 ± 1.9 pmol/10^6 cells/10 min, indicative of a high affinity, low capacity system. However, the uptake of MIBG appeared to be more complex (Fig. 2B). The total uptake took the form of a straight line (r = 0.9992 between 10 and 50 μM) superimposed upon a hyperbola. This suggested that uptake was composed of a saturable and an unsaturable component. Extrapolation of the linear component to 0 μM, and subtraction of this component from total uptake, revealed a high affinity, saturable component of MIBG uptake. From this derived, saturable component, the kinetic parameters of the high affinity system were calculated using an appropriate linear transformation (21). Using this technique, a small, linear component of NE uptake could also be subtracted from total uptake. The kinetic parameters for MIBG (n = 2) and NE (n = 4) were similar although not identical. Specifically, MIBG had a similar K_m (1.22 ± 0.12 versus 1.41 ± 0.50 μM; $p < 0.05$) but a higher V_m (64.3 ± 3.3 versus 36.6 ± 7.2 pmol/10^6 cells/10 min; $p < 0.05$). These kinetic experiments suggested that the uptake of NE and MIBG were the sum of two systems, one saturable and the other unsaturable.

Sodium dependency of uptake. The equimolar substitution of LiCl for NaCl is a classic means of demonstrating the sodium dependency and sodium specificity of the Uptake$_1$ system (22, 23). In preliminary experiments, we observed that LiCl substitution did not completely inhibit the uptake of either NE and MIBG, a response inconsistent with the absolute sodium dependency and sodium specificity of the Uptake$_1$ mechanism (24, 25). In addition, the uptake of each agent was inhibited to a different degree. Since the biphasic uptake of each agent might be linked to its relative sodium dependency of uptake, we evaluated the uptake of each agent, over a wide range of concentrations, in H-KRG solution and zero-sodium H-KRG solution.

Sodium-independent uptake may be defined as uptake which occurred in the zero-sodium H-KRG buffer. As a corollary, sodium-dependent uptake is then defined as uptake in the presence of NaCl less the sodium-independent uptake, i.e., uptake in zero-sodium H-KRG solution. Total uptake is thus the uptake in H-KRG, which is the sum of the sodium-dependent and sodium-independent components.

As shown in Fig. 3, the relative percentage of these two uptake components was a function of NE and MIBG concentration. In general, both agents, at low concentrations, entered primarily by the sodium-dependent uptake system. At high concentrations, the sodium-independent uptake system was more important. Specifically, NE uptake was approximately 90% sodium-dependent at low concentrations (0.5 to 2.0 μM), but was increasingly sodium-independent at higher concentrations. At 20 μM NE, the uptake was approximately 50% sodium-independent. MIBG uptake was approximately 60% sodium-dependent at 0.5 μM but virtually sodium-independent at 20 μM. Additional kinetic studies carried out to 5 mM with both NE and MIBG demonstrated sodium-independent uptake to be an unsaturable process; the sodium-independent uptake was linear with respect to either NE concentration (r = 0.98) or MIBG concentration (r = 0.98) (data not shown).

Ouabain inhibition studies. Ouabain, a (Na^+/K^+)-ATPase inhibitor (23), should inhibit only the sodium-dependent uptake component of total uptake. The effect of

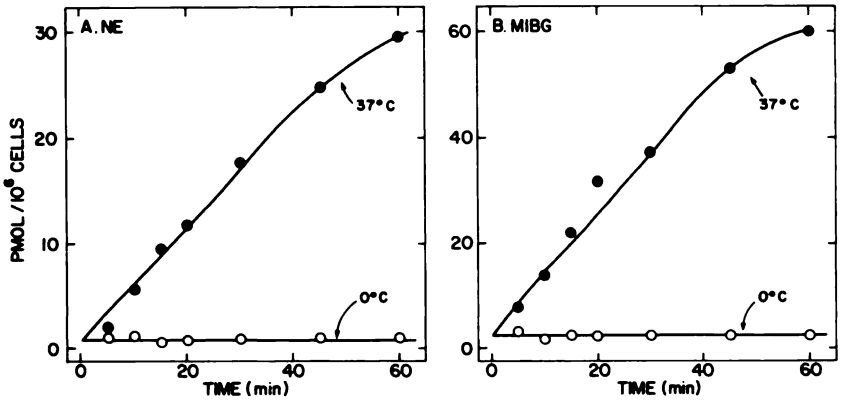

FIG. 1. *Time and temperature dependence of apparent uptake into cultured bovine adrenomedullary cells*

A, 0.25 μM [^{3}H]NE; B, 0.25 μM [^{125}I]MIBG. Adrenomedullary cells were incubated at 37° (●) or 0° (○). Uptake was terminated at various times by removal of the incubation medium and extraction from the cells as described in Materials and Methods. Uptake is expressed as picomoles/10^6 cells.

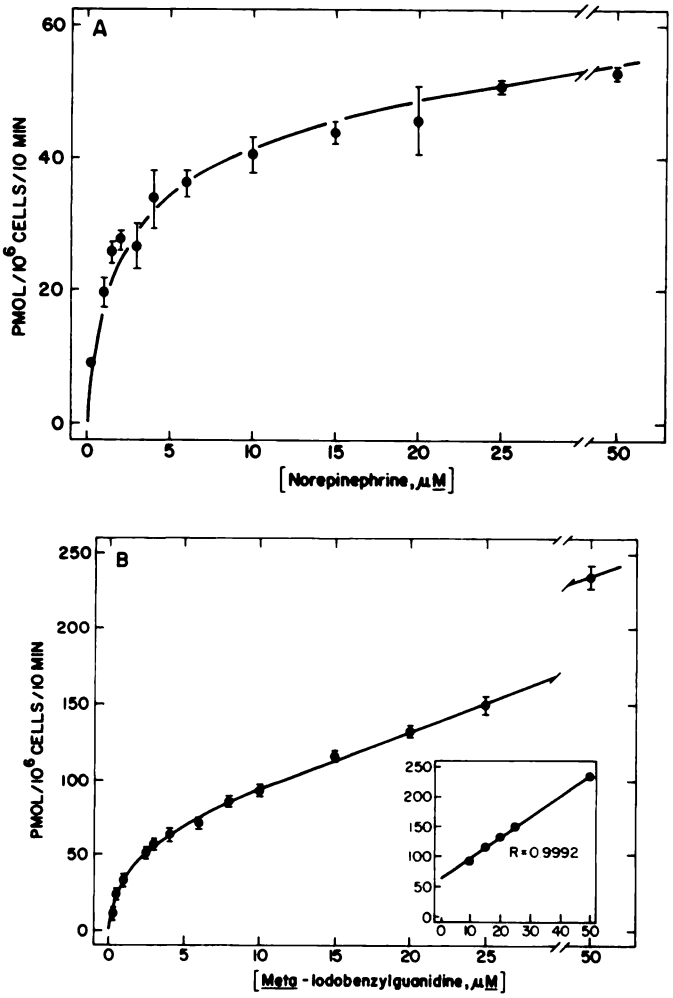

FIG. 2. *Total uptake of [^{3}H]NE (A) and [^{125}I]MIBG (B) into cultured bovine adrenomedullary cells as a function of concentration*

Adrenomedullary cells were incubated with increasing concentrations (0.25–0.50 μM) of [^{3}H]NE or [^{125}I]MIBG at 37° and 0° described in Materials and Methods. Total uptake (●) was calculated as apparent uptake at 37° (quadriplicate values) less uptake at 0° (duplicate values) and is expressed as picomoles/10^6 cells/10 min. The linear uptake of MIBG between 10 and 50 μM is shown in the inset.

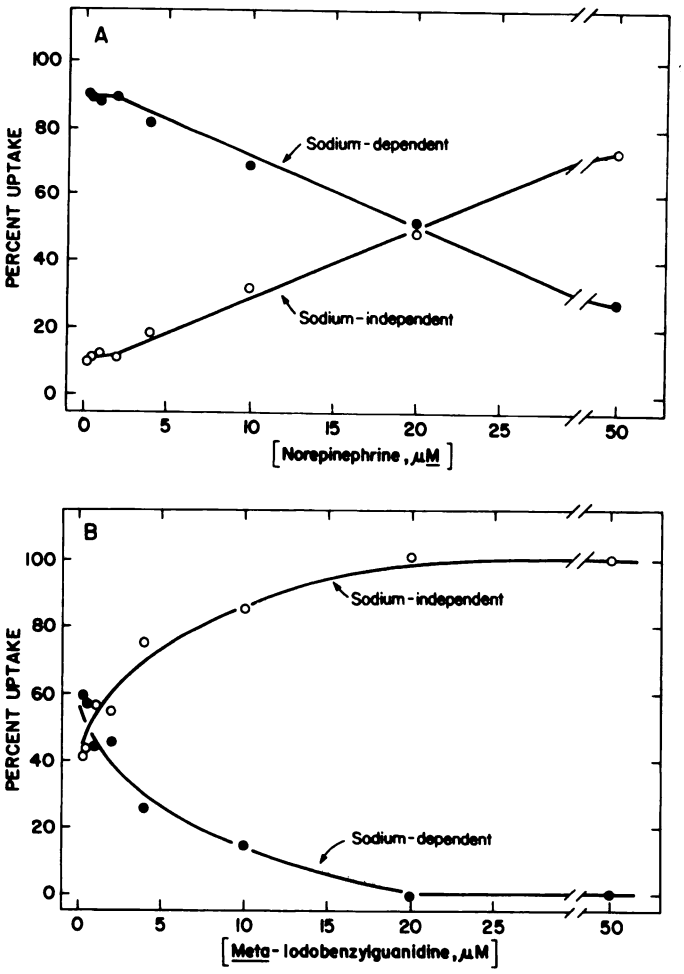

FIG. 3. *Percentage of the total uptake for the sodium-dependent and sodium-independent uptake systems of [^{3}H]NE (A) and [^{125}I]MIBG (B) as a function of concentration.*

The percent sodium-independent uptake (○) represents uptake in zero-sodium H-KRG expressed as a percentage of the total uptake (uptake in H-KRG). The percent sodium-dependent uptake (●) is the total uptake (uptake in H-KRG) minus the uptake in zero-sodium H-KRG, expressed as a percentage of the total uptake.

ouabain on the uptake of 0.5 and 20 μM NE and MIBG in H-KRG solution and zero-sodium H-KRG solution is shown in Figs. 4 and 5. We selected 0.5 and 20 μM NE and MIBG because these concentrations enabled us to examine the sodium-dependent and sodium-independent uptake systems in isolation or in combination. The sodium-dependent uptake of 0.5 μM NE was ouabain-sensitive (Fig. 4A) while the sodium-independent uptake of 0.5 μM NE, which was 10 to 15% of the total uptake, was not ouabain-sensitive (data not shown). The sodium-dependent uptake of 0.5 μM MIBG was also ouabain-sensitive; the sodium-independent uptake of 0.5 μM MIBG was ouabain-insensitive (Fig. 4B). In agreement with the observations at the lower concentrations, ouabain inhibited only the sodium-dependent component of uptake at 20 μM NE or MIBG; it had no effect upon sodium-independent uptake of either 20 μM NE or MIBG (Fig. 5). In each experiment, however, there appeared to be a small fraction of the total uptake, approximately 10%, which was sodium-dependent but ouabain-insensitive.

Since the action of ouabain is indirect via an effect on the (Na^+/K^+)-ATPase, the ouabain inhibition of the sodium-dependent component should be a time-dependent process. As shown in Fig. 6, ouabain inhibition of the sodium-dependent uptake of both 0.5 μM NE and 0.5 μM MIBG was dependent on the preincubation time with ouabain. The small fraction of total uptake which appeared to be sodium-dependent but ouabain-insensitive (Figs. 4 and 5) was most likely the result of insufficient time of cell exposure to ouabain since the ouabain was added in combination with either [^{3}H]NE or [^{125}I]MIBG.

Metabolic inhibition studies. The energy requirements of the sodium-dependent and sodium-independent uptake components for both NE and MIBG were evaluated in the presence or absence of glucose with or without 5 mM 2-deoxy-D-glucose and/or 10 mM sodium azide (Table 1). The sodium-dependent uptake of each agent was inhibited whereas the sodium-independent uptake of

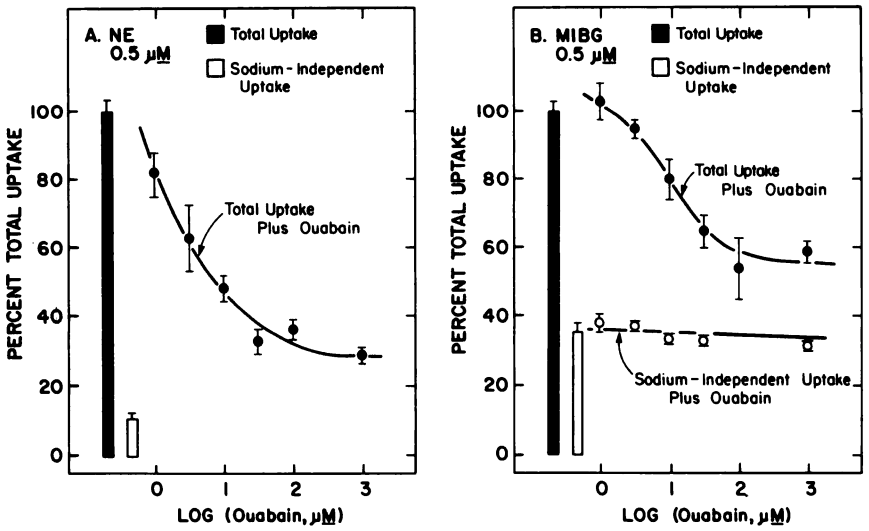

FIG. 4. *Selective inhibition of the sodium-dependent uptake system for 0.5 μM [^{3}H]NE (A) and 0.5 μM [^{125}I]MIBG (B) by ouabain, a (Na^+/K^+)-ATPase inhibitor*

Sodium-independent uptake (uptake in zero-sodium H-KRG, □) is expressed as a percentage of the total uptake (uptake in H-KRG, ■). Ouabain (1.0 to 1000 μM) was incubated concomitantly with either [^{3}H]NE or [^{125}I]MIBG in zero-sodium H-KRG (○) or in H-KRG (●) and uptake under these conditions is expressed as a percentage of the total uptake. Ouabain inhibited ($p < 0.05$) total uptake of 0.5 μM NE at ≥1.0 μM and total uptake of 0.5 μM MIBG at ≥10.0 μM. Ouabain, at concentrations between 1 and 1000 μM, did not inhibit ($p > 0.05$) uptake of 0.5 μM NE (data not shown) or 0.5 μM MIBG in the zero-sodium buffer.

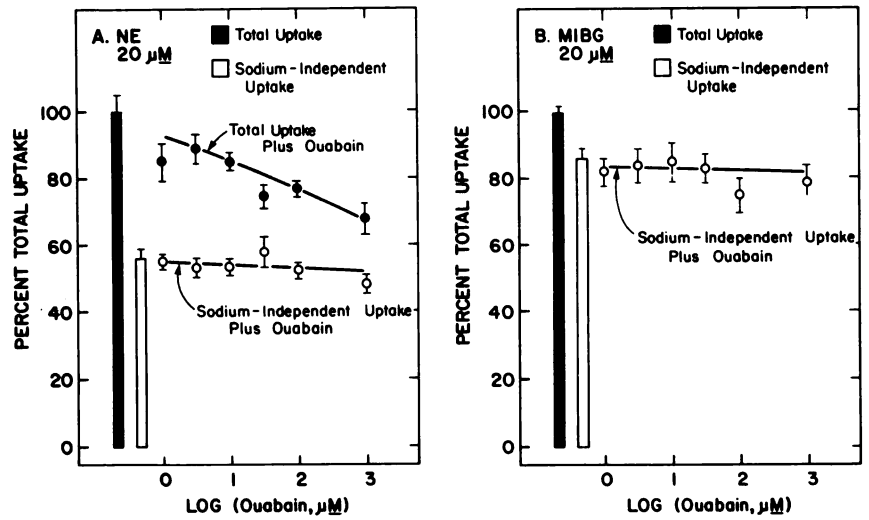

FIG. 5. *Selective inhibition of the sodium-dependent uptake system for 20 μM [^{3}H]NE (A) and 20 μM [^{125}I]MIBG (B) by ouabain, a (Na^+/K^+)-ATPase inhibitor*

Sodium-independent uptake (uptake in zero-sodium H-KRG, □) is expressed as a percentage of the total uptake (uptake in H-KRG, ■). Ouabain (1.0 to 1000 μM) was incubated concomitantly with either [^{3}H]NE or [^{125}I]MIBG in zero-sodium H-KRG (○) or in H-KRG (●) and uptake under these conditions is expressed as a percentage of the total uptake. Ouabain inhibited ($p < 0.05$) total uptake of 20 μM NE at ≥1.0 μM and total uptake of 20 μM MIBG at ≥10 μM (data not shown). Ouabain, at concentrations between 1 and 1000 μM, did not inhibit the uptake of 20 μM NE or 20 μM MIBG in the zero-sodium buffer.

each agent was not inhibited or inhibited to a small degree. Specifically, sodium azide was a more effective inhibitor of the sodium-dependent uptake component than 2-deoxy-D-glucose. The inhibition of the sodium-dependent uptake of NE and MIBG by sodium azide and 2-deoxy-D-glucose was more marked in glucose-deprived medium. The sodium-dependent uptake of each was further depressed when these metabolic inhibitors were present in the incubation mixture as well as the preincubation mixture.

Competitive inhibition of uptake. To determine if the sodium-dependent uptake component is a shared system for NE and MIBG, we evaluated the ability of each agent to inhibit the sodium-dependent uptake of the other. MIBG inhibited the sodium-dependent uptake of 0.5 μM NE with an IC_{50} of 1.6 μM, and NE inhibited the sodium-dependent uptake of 0.5 μM MIBG with an IC_{50} of 2.4 μM.

DISCUSSION

These studies demonstrate that two different uptake systems exist for both NE and MIBG in cultured bovine

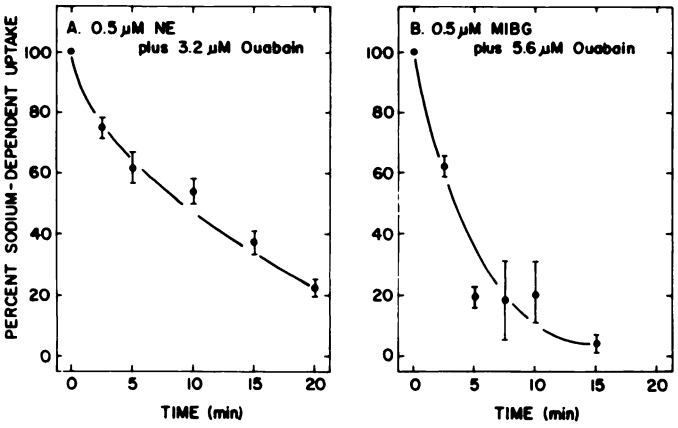

FIG. 6. *Time dependence of the ouabain inhibition of the sodium-dependent uptake of NE and MIBG*

A, 0.5 μM [^{3}H]NE plus 3.2 μM ouabain; B, 0.5 μM [^{125}I]MIBG plus 5.6 μM ouabain. The sodium-dependent uptake was determined from the total uptake (uptake in H-KRG) minus the uptake in zero-sodium H-KRG. Uptake was measured as described in Materials and Methods after preincubating the cells with ouabain for various times.

adrenomedullary cells. The existence of two uptake systems was initially predicted by the uptake kinetic studies (Fig. 2). However, the identification of two distinct systems was made using an equimolar substitution of LiCl for NaCl to demonstrate their sodium dependency (Fig. 3). Although LiCl can have other cellular actions (26–28), the use of LiCl to discriminate a sodium-dependent uptake system from a sodium-independent uptake system was confirmed by the ouabain inhibition studies (Figs. 4, 5, and 6). Ouabain, a (Na^+/K^+)-ATPase inhibitor (23, 29), selectively inhibited the sodium-dependent uptake of NE and MIBG in a dose-dependent and time-dependent manner. The inhibition of the sodium-dependent uptake of both agents by ouabain was evident whether the sodium-dependent uptake was a major (Fig. 4) or a minor (Fig. 5) component of the total uptake. In contrast, the inability of ouabain, even at the 1 mM level, to suppress the sodium-independent uptake of either agent further suggests that the sodium-dependent uptake system and the sodium-independent uptake system are separate and distinct.

The basis for the clearly biphasic pattern of MIBG uptake (Fig. 2B) was apparent when saturation studies were performed in H-KRG and zero-sodium H-KRG (Fig. 3B). MIBG uptake became predominantly sodium-independent (80 to 100%) at about 10 μM, the same concentrations at which the kinetic plot assumed a linear and apparently unsaturable character. At concentrations less than 10 μM, the sodium-independent uptake system was not obvious because the contribution of the high affinity, sodium-dependent uptake system predominated.

NE uptake (Figs. 2A and 3A) was also composed of a sodium-dependent uptake system and sodium-independent uptake system. The smaller contribution of the sodium-independent uptake to the total uptake may explain the apparent saturability of NE uptake. This saturation was not unequivocal since there was a slight increase in velocity from 25 to 50 μM such that a linear uptake component could be derived. We did not observe an abrupt, sigmoidal transition from a high affinity system to the dominant, low affinity, unsaturable system at 1 μM as reported by Kenigsberg and Trifaro (14). This difference is probably related to the differences between our more conventional uptake technique (30) and the "peeling technique" (14) which would appear to enhance the contribution of the apparently unsaturable (sodium-independent) system.

Uptake studies with and without the metabolic inhibitors 2-deoxy-D-glucose and sodium azide demonstrated that the sodium-dependent uptake system was clearly energy-dependent and, therefore, an active transport process (Table 1). In contrast, the sodium-independent uptake system appears not to be an energy-dependent process. As seen for other systems (31), the energy requirements for the sodium-dependent uptake system may be provided by several routes; extreme conditions of continuous exposure to both 2-deoxy-D-glucose and sodium azide in combination with glucose deprivation

TABLE 1

Effect of metabolic inhibitors on the sodium-dependent and sodium-independent uptake of [^{3}H]NE and [^{125}I]MIBG into cultured bovine adrenomedullary cells

Cells were preincubated at 37° for 30 min in zero-sodium H-KRG or H-KRG in the presence or absence of glucose with or without 5 mM 2-deoxy-D-glucose and/or 10 mM sodium azide. After washing the cells with the identical medium but without 2-deoxyglucose and/or sodium azide, the cells were incubated in the identical medium containing 0.5 and 20 μM of either [^{3}H]NE or [^{125}I]MIBG to measure uptake. Results were expressed as a percentage, mean ± standard deviation of total uptake.

± Glucose	Agents	Percentage of sodium-dependent uptake		Percentage of sodium-independent uptake	
		0.5 μM NE	0.5 μM MIBG	20 μM NE	20 μM MIBG
+	None	100	100	100	100
−	None	83.7 ± 7.8	57.2 ± 9.3	102 ± 13.2	94.1 ± 8.2
+	2-Deoxyglucose	96.8 ± 10.3	84.4 ± 9.7	100 ± 9.0	80.1 ± 8.8
+	Sodium azide	79.6 ± 2.0	73.8 ± 4.1	97.2 ± 4.7	97.1 ± 6.9
−	Sodium azide	40.7 ± 0.4	55.9 ± 4.2	99.8 ± 3.5	85.9 ± 4.6
−	2-Deoxyglucose + sodium azide	22.6 ± 4.6	45.9 ± 2.8	85.1 ± 7.6	87.1 ± 4.2
−	2-Deoxyglucose + sodium azide[a]	12.3 ± 1.1	11.8 ± 4.7	101 ± 5.7	93.3 ± 4.1

[a] These cells were preincubated and incubated in the presence of 5 mM 2-deoxyglucose and 10 mM sodium azide.

were necessary to achieve almost 90% inhibition of uptake.

These experiments indicate that both NE and MIBG are transported into bovine adrenomedullary cells by a sodium-dependent uptake system that was characterized by: 1) temperature dependency; 2) high affinity: K_m of 1.22 ± 0.12 μM for MIBG and 1.41 ± 0.50 μM for NE; 3) low capacity: V_m of 64.3 ± 3.3 pmol/10^6 cells/10 min for MIBG and 36.6 ± 7.2 pmol/10^6 cells/10 min for NE; 4) saturability; 5) ouabain sensitivity; and 6) energy dependency. The sodium-dependent uptake system for both agents fulfills many of the criteria for the neuronally characterized $Uptake_1$ system (25, 31–34). Since both NE and MIBG can inhibit the sodium-dependent uptake of the other, these two agents appear to be transported by the same carrier. Additionally, these studies suggest that NE is transported as the cationic species since MIBG will always be protonated.

Both NE and MIBG are also transported into bovine adrenomedullary cells by a sodium-independent uptake system that was characterized by: 1) temperature dependency; 2) unsaturability out to 5 mM; 3) ouabain insensitivity; and 4) energy independency. The characteristics of the sodium-independent uptake system are consistent with a diffusion mechanism (21) and/or an interaction with membrane phospholipids (35). The sodium-independent uptake system is most likely a passive diffusion process since the methodology used demonstrated increased uptake as substrate concentration increased.

In summary, using cultured bovine adrenomedullary cells as a model system, we have shown that both NE and MIBG may be transported by two different pathways which differ by their sodium dependency and sodium specificity. The fundamental difference between the *in vitro* uptake of NE and MIBG appears to be the degree, at any given concentration, to which sodium-independent uptake predominated. This may be based on the more lipophilic cationic character of MIBG.

Although the *in vivo* milieu is more complex than our *in vitro* model, extrapolated blood concentrations of MIBG in patients, receiving either imaging or therapy doses of [^{131}I]MIBG, suggest that scintiscans of human adrenals and pheochromocytomas reflect uptake predominantly by the sodium-dependent pathway (3–12). In addition to its diagnostic and therapeutic value, it appears that MIBG may prove to be a uniquely useful tool to probe adrenomedullary physiology both *in vivo* and *in vitro*.

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