Sympathomimetic Effects of MIBG: Comparison with Tyramine

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Because nothing is known about whether metaiodobenzylguanidine (MIBG) has tyramine-like actions, the sympathomimetic effects of MIBG were determined in the isolated rabbit heart and compared with those of tyramine. **Methods:** Spontaneously beating rabbit hearts were perfused with Tyrode's solution (Langendorff technique; 37°C; 26 mL/min), and the heart rate as well as the norepinephrine and dopamine overflow into the perfusate was measured before and after doses of MIBG or tyramine (0.03–10 μmol) given as bolus injections (100 μL) into the aortic cannula. K_m and V_{max} values for the neuronal uptake ($uptake_1$) of ^{125}I-MIBG and ^{14}C-tyramine were obtained in human neuroblastoma (SK-N-SH) cells. The K_i of MIBG for inhibition of the ^{3}H-catecholamine uptake mediated by the vesicular monoamine transporter was determined in membrane vesicles obtained from bovine chromaffin granules and compared with the previously reported K_i value for tyramine determined under identical experimental conditions. **Results:** By producing increases in heart rate and norepinephrine overflow, both compounds had dose-dependent sympathomimetic effects in the rabbit heart. MIBG was much less effective than tyramine in increasing heart rate (maximum effect 59 versus 156 beats/min) and norepinephrine overflow (maximum effect 35 versus 218 pmol/g). Tyramine also caused increases in dopamine overflow, whereas MIBG was a poor dopamine releaser. At a dose of 10 μmol, the increase in heart rate lasted more than 60 min after MIBG and about 20 min after tyramine injection. Accordingly, the norepinephrine overflow caused by 10 μmol MIBG and tyramine declined with half-lives of 57.8 and 2.2 min, respectively. The effects of both drugs were drastically reduced in hearts exposed to 2 μmol/L desipramine. The kinetic parameters characterizing the saturation of neuronal uptake by ^{125}I-MIBG and ^{14}C-tyramine were similar for the two compounds: K_m values of MIBG and tyramine were 1.6 and 1.7 μmol/L, respectively, and V_{max} values of MIBG and tyramine were 43 and 37 pmol/mg protein/min, respectively. However, in inhibiting the vesicular ^{3}H-catecholamine uptake, MIBG was eight times less potent than tyramine. **Conclusion:** MIBG is much less effective than tyramine as an indirect sympathomimetic agent. This is probably a result of its relatively low affinity for the vesicular monoamine transporter and explains the relatively poor ability of the drug to mobilize norepinephrine stored in synaptic vesicles. The long duration of MIBG action results primarily from the drug not being metabolized by monoamine oxidase. The sympathomimetic effects of MIBG described here are not likely to come into play in patients given diagnostic or common therapeutic doses of radioiodinated MIBG.

Key Words: metaiodobenzylguanidine; tyramine; indirect sympathomimetic action; neuronal uptake; vesicular monoamine transporter

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Because metaiodobenzylguanidine (MIBG) is taken up by and concentrated in tumors originating from the sympathetic nervous system, radioiodinated MIBG is used widely to diagnose and treat these disorders. Several aspects of the pharmacology of MIBG have been reviewed (*1–3*). MIBG acts as an alternative substrate of the neuronal norepinephrine transporter (neuronal uptake [$uptake_1$], according to Iversen [*4*]) and is therefore avidly taken up by postganglionic sympathetic neurons (*5,6*) and cells derived from these neurons, including adrenomedullary chromaffin (*7,8*), human neuroblastoma (SK-N-SH) (*9,10*) and rat pheochromocytoma (PC-12) cells (*10*). After $uptake_1$, MIBG is further accumulated within transmitter storage vesicles because it is also a substrate for the reserpine-sensitive vesicular monoamine transporter (*11*). Other membrane-associated amine transporters may likewise contribute to the distribution of MIBG in vivo. For example, the extraneuronal monoamine transporter ($uptake_2$, according to Iversen [*4*]) is responsible for MIBG uptake by non-neuronal cells in the rat heart (*12*), and MIBG uptake observed in endothelial cells of the pulmonary circulation (*13*) and blood platelets (*14*) is mediated by norepinephrine or serotonin transporters known to operate in the plasma membrane of these cells. As expected from its chemical structure, MIBG does not appear to interact with α- or β-adrenoceptors and is metabolized neither by monoamine oxidase nor by catechol-O-methyltransferase (*15,16*).

Other aspects of the pharmacology of MIBG are less well described. For instance, despite the fact that indirectly acting sympathomimetic amines and MIBG have certain properties in common (e.g., both are good substrates for $uptake_1$ and the reserpine-sensitive vesicular monoamine transporter), the possibility that MIBG releases norepinephrine and exerts indirect sympathomimetic effects has not been explored in

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detail. Therefore, spontaneously beating, isolated, perfused rabbit hearts were used to compare the abilities of MIBG and tyramine, the prototype of indirectly acting amines, to induce norepinephrine release and increases in heart rate. We also compared the saturation kinetics for MIBG and tyramine as substrates of uptake$_1$ and as inhibitors of the reserpine-sensitive vesicular monoamine transporter.

MATERIALS AND METHODS

Materials

MIBG was synthesized as described by Wieland et al. (*17*). Their method was slightly modified; MIBG was crystallized as acetate salt and not as sulfate salt. As determined by high-performance liquid chromatography (HPLC) and ultraviolet detection, the purity of the drug was >99%.

^{125}I-MIBG was synthesized by a Cu(I)-assisted, nonisotopic exchange method (*18*). Sodium disulfite was added to 40–80 MBq ^{125}I (Amersham, Braunschweig, Germany), and the solution evaporated to dryness. Meta-bromobenzylguanidine (purity > 99%) and CuCl, both dissolved in acetic acid, were added and the mixture heated to 180°C for 10 min. Then, the acetic acid was evaporated, and the residue was dissolved in eluent and subjected to HPLC. Separation was achieved on a Purospher column RP-18 (5 µm; 250 × 4 mm) (Merck, Darmstadt, Germany) with 0.01 mol/L NaH_2PO_4/CH_3CN (950/50; v/v) as eluent. The fraction containing ^{125}I-MIBG was collected, the eluent evaporated and the product dissolved in isotonic phosphate buffer (pH 7.4). The specific activity of ^{125}I-MIBG was determined to be 81.4 MBq/µmol.

Other drugs used in the study were desipramine hydrochloride, dopamine hydrochloride, (-)-norepinephrine bitartrate, nisoxetine hydrochloride, pargyline hydrochloride, reserpine, tyramine hydrochloride (Sigma, Deisenhofen, Germany), 3′,4′-dihydroxy-2-methylpropiophenone (Upjohn, Kalamazoo, MI), heparin (Liquemin; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany), ^{3}H-(ring-2,5,6)-(-)-norepinephrine (specific activity 2024 GBq/mmol), ^{14}C-tyramine (specific activity 1672 GBq/mmol) (NEN-DuPont, Dreieich, Germany) and Eagle's minimum essential medium, fetal calf serum (Gibco-Life Technologies, Karlsruhe, Germany).

Stock solutions of MIBG acetate (100 mmol/L) and tyramine hydrochloride (300 mmol/L) were prepared in deionized water. On the day of the experiment, the dilutions were made in saline.

Isolated Perfused Rabbit Hearts

Rabbits (1.8–2.3 kg) of either sex and mixed strains were used. After intravenous administration of heparin (500 U) and an overdose of sodium pentoparbitone (80 mg/kg), they were killed by exsanguination. Hearts were quickly removed, the aorta cannulated and the coronary vessels perfused (Langendorff technique) with modified Tyrode's solution of the following composition: 118 mmol/L NaCl, 4.5 mmol/L KCl, 25 mmol/L $NaHCO_3$, 1.4 mmol/L NaH_2PO_4, 1.2 mmol/L $MgSO_4$, 1.4 mmol/L $CaCl_2$ and 11 mmol/L glucose. The perfusion medium was maintained at 37°C and saturated with 5% CO_2 in O_2 (resulting in a pH of 7.4); a roller pump was used to deliver it at a constant rate of 26 mL/min. The coronary perfusion pressure and the left ventricular pressure of the spontaneously beating heart were monitored with Isotec pressure transducers (Hugo Sachs Elektronic, March-Hugstetten, Germany) connected to a side arm of the aortic cannula and a saline-filled, latex balloon inserted into the left ventricle, respectively. The heart rate and the left ventricular pressure amplitude were derived from the left ventricular pressure signal and recorded by a computer-assisted signal processing system (MacLab system and Chart program; ADInstruments, Castle Hill, New South Wales, Australia). After an equilibration period of 30 min, MIBG, tyramine or vehicle (solvent of the drugs) was given as a bolus injection (100 µL) into the aortic cannula. In some experiments, the uptake$_1$ inhibitor desipramine (2 µmol/L) was present in the perfusion medium throughout.

Three groups of experiments were performed. Group I served to determine dose-response curves for the heart-rate-increasing as well as the norepinephrine- and dopamine-releasing effects of MIBG and tyramine. To this end, MIBG or tyramine doses of 0.03, 0.1, 0.3, 1, 3 and 10 µmol were administered in that order consecutively at intervals of 10–35 min, and heart rate and left ventricular pulse pressure (LVPP), a measure of contractility, were monitored. The heart rate response to MIBG or tyramine was defined as the increase in heart rate above the baseline value observed before the injection of the first drug dose. To determine the overflow of endogenous norepinephrine and dopamine into the perfusion medium, the venous effluent from the heart was collected continuously at 5-min intervals and analyzed for catecholamines. After injection of MIBG or tyramine doses of 0.03, 0.1, 0.3, 1, 3 and 10 µmol, the number of samples collected at 5-min intervals was 2, 3, 4, 5, 7 and 9, respectively. A 5-min collection period before the first dose was used to obtain baseline values. The norepinephrine and dopamine overflow observed after MIBG or tyramine injection was corrected for the spontaneous (baseline) overflow. Because the solubility of MIBG was limited (concentration in stock solution 100 mmol/L), the highest MIBG (and tyramine) dose used was 10 µmol (i.e., 100 µL MIBG stock solution).

Group II served to determine effects of a single dose of 10 µmol MIBG or tyramine both in the absence and presence of 2 µmol/L desipramine. After drug administration, heart rate and catecholamine overflow were monitored in these experiments for 60 min. Group III experiments were used to study the effects of vehicle solutions (i.e., 100 µL distilled water, saline or 100 mmol/L sodium acetate) on heart rate and LVPP. The effect of 100 µL of 100 mmol/L sodium acetate on LVPP was also determined in hearts paced at 300 beats/min (field stimulation at a voltage of 9 V with square pulses of 3-ms width delivered at a frequency of 5 Hz).

For chemical analysis of norepinephrine and dopamine, 10-mL portions of the perfusate samples were first mixed (rotatory mixer, 5 min) with 1 ng dihydroxybenzylamine (internal standard), 500 µL Tris[hydroxymethyl]aminomethane (TRIS)-HCl buffer (2 mol/L, pH 8.7) and 40 mg Al_2O_3 and then filtered (GF 52; Schleicher & Schuell, Dassel, Germany). The Al_2O_3 remaining on the filter was washed twice with 1 mL distilled water and then eluted twice with 100 µL $HClO_4$ (0.1 mol/L), respectively. After pooling of the two eluate fractions, 100 µL of the eluate was subjected to HPLC. The HPLC system consisted of a type 515 pump, an automatic sample injector WISP 717 (Waters, Eschborn, Germany) and a Coulochem II electrochemical detector connected to a triple electrode system (cell models 5021 and 5011; ESA, Bedford, MA). The data were sampled and processed by the Millennium 2010 Chromatography-Manager (Waters). For the chromatographic separation of norepinephrine and dopamine, a 5-µm Nucleosil 100 C_{18} column (125 × 3 mm internal diameter; Macherey-Nagel, Düren, Germany), maintained at 25°C, was used. The mobile phase (50 mmol/L KH_2PO_4, 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.3 mmol/L sodium octanesulfonate and 2% [v/v] methanol, pH 3.0)

was pumped at a flow rate of 0.8 mL/min. The potential of the first electrode in the series was set to 350 mV, the second to 300 mV and the third to −350 mV versus the internal solid state palladium reference electrode. The mean 3,4-dihydroxybenzylamine hydrobromide (DHBA) recovery from the perfusate amounted to 72%; this mean value had an intra-assay coefficient of variation of 4% and an interassay coefficient of variation of 7%. The results concerning norepinephrine and dopamine were corrected for the DHBA recovery.

Human Neuroblastoma (SK-N-SH) Cells

The human neuroblastoma cell line SK-N-SH (subtype SY5Y; American Type Culture Collection, Rockville, MD) was used to compare the saturation kinetics of MIBG, tyramine and norepinephrine uptake mediated by uptake$_1$. Cells were cultured in tissue culture dishes (60-mm diameter) with Eagle's minimum essential medium supplemented with 10% fetal calf serum. Initial rates of uptake of ^{125}I-MIBG, ^{14}C-tyramine and ^{3}H-norepinephrine were determined at 37°C in N-[2-hydroxyethyl]piperazine-N′-[2-ethane sulfonic acid] (HEPES)-buffered Krebs-Ringer solution (KRS) of the following composition: 125 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 1.3 mmol/L $CaCl_2$, 25 mmol/L HEPES, 5.55 mmol/L glucose and 1.0 mmol/L ascorbic acid. Catechol-O-methyltransferase and monoamine oxidase were inhibited by the presence of 10 μmol/L 3′,4′-dihydroxy-2-methylpropiophenone and 10 μmol/L pargyline, respectively. After 1 min of incubation with the labeled amines, cells were washed three times with 2 mL ice-cold KRS and then solubilized in 1.5 mL of either 0.3 mol/L NaOH (^{125}I-MIBG) or 0.1% TritonX100 (^{14}C-tyramine and ^{3}H-norepinephrine). The amount of radioactivity in 1 mL of solubilzed cells was determined in a gamma counter (^{125}I-MIBG) or a liquid scintillation counter (^{14}C-tyramine and ^{3}H-norepinephrine). The protein content of the solubilized cells was quantified by the method of Lowry et al. (*19*). To increase the substrate concentration of ^{125}I-MIBG (0.1, 0.2, 0.5, 1.5, 5 and 10 μmol/L) and ^{3}H-norepinephrine (0.03, 0.1, 0.3, 1, 3 and 10 μmol/L) in the incubation medium, the specific activities of the labeled amines were lowered by addition of unlabeled amines. On the other hand, when the concentration of ^{14}C-tyramine was increased (0.5, 1, 1.5, 3, 6 and 10 μmol/L), the specific activity remained unchanged.

Initial rates of the uptake of ^{125}I-MIBG, ^{14}C-tyramine and ^{3}H-norepinephrine were always determined in the absence (total uptake) and presence (nonmediated uptake) of 10 μmol/L nisoxetine (a selective uptake$_1$ blocker), and the saturable uptake mediated by uptake$_1$ was calculated from the difference between total and nonmediated uptake.

Membrane Vesicles of Bovine Chromaffin Granules

Chromaffin granules were prepared from bovine adrenomedullary tissue by density gradient centrifugation over 1.6 mol/L sucrose. Membrane vesicles were obtained after lysis of chromaffin granules (10 min at 30°C, pH 6.0) in a medium containing 180 mmol/L urea, 20 mmol/L ascorbic acid, 10 mmol/L K_2HPO_4/KH_2PO_4 and 0.5 mmol/L EDTA. After sedimentation (144,000*g* for 20 min), membrane vesicles were resuspended in 270 mmol/L sucrose/10 mmol/L TRIS-H_2SO_4 (pH 6.0) and stored at −70°C. One milliliter of this vesicle suspension contained approximately 1 mg protein and 60–100 nmol endogenous epinephrine/norepinephrine with a typical mixing ratio of 7:3. Before use, the pH of the freshly thawed vesicle suspension was adjusted to 7.3 with 1 mol/L TRIS.

Uptake experiments were performed at a pH of 7.3 and at 30°C. The 5-mL incubation mixture contained 0.5 mL of the membrane vesicle suspension as well as 330 mmol/L sucrose, 30 mmol/L TRIS-HCl (pH 7.3) and 3 mmol/L adenosine triphosphate-$MgSO_4$. After 10 min of preincubation in the absence or presence of 0.1 μmol/L reserpine, a selective inhibitor of the vesicular monoamine transporter, and various concentrations of MIBG (3–1000 μmol/L), the uptake was started by the addition of a ^{3}H-catecholamine mixture (70% epinephrine plus 30% norepinephrine plus trace amounts of ^{3}H-norepinephrine) to give final total catecholamine concentrations of 13, 31 or 108 μmol/L. After 3 min of incubation, a 4-mL aliquot of the incubation mixture was transferred into large ice-cold beakers containing 400 mmol/L sucrose and filtered through cellulose nitrate membrane filters (0.45 μm). After being washed with ice-cold sucrose, the filters were dried (80°C, 30 min), dissolved in scintillation cocktail and analyzed for radioactivity by liquid scintillation counting. A 0.5-mL aliquot of the incubation mixture was centrifuged (144,000*g* for 20 min). The supernatant served to determine the specific activity in the incubation medium (*20,21*), and the pellet was used for protein determination (*19*).

To measure the reserpine-sensitive uptake that reflects the uptake component mediated by the vesicular monoamine transporter, the vesicular ^{3}H-catecholamine uptake was always determined in the absence and presence of 0.1 μmol/L reserpine. As shown previously for identical experimental conditions, the reserpine-sensitive vesicular ^{3}H-catecholamine uptake proceeds at initial rates for at least 3 min, is saturable and exhibits (at a pH of 7.3) a K_m of approximately 10 μmol/L and a V_{max} of 10–20 nmol/mg protein/min (*20,21*).

Data Analysis and Statistics

Dose- and concentration-response curves for the various effects of MIBG or tyramine were analyzed by fitting Hill's equation with a nonlinear least squares method to the experimental results. Hill's equation of the form $E = E_{max}\ D^{nH}/(EC_{50}{}^{nH} + D^{nH})$ (E_{max}, maximum effect; EC_{50}, drug dose producing $E_{max}/2$; nH, apparent Hill coefficient = midpoint slope of the curve) was used to analyze dose-response curves showing increases in heart rate and catecholamine overflow (E) in response to the drug dose (D). Similarly, the equation used to analyze concentration-response curves relating the vesicular ^{3}H-catecholamine uptake (U, expressed as percentage of control uptake) to the concentration of MIBG (C) had the form $U = I_{max}\ C^{nH}/(IC_{50}{}^{nH} + C^{nH})$ (I_{max}, maximum inhibition of uptake; IC_{50}, MIBG concentration producing $I_{max}/2$). K_i values were calculated from IC_{50} values, as described by Cheng and Prusoff (*22*). The Michaelis-Menten equation was fitted to the saturation curves relating initial rates of uptake to substrate concentrations (i.e., to the results obtained in SK-N-SH cells) by nonlinear regression analysis to give values of K_m and V_{max}.

Results presented are either arithmetic mean ± SEM or geometric mean with 95% confidence limits; n is the number of experiments. The statistical evaluation of differences between means (Student *t* test; analysis of variance followed by the Bonferroni test for multiple comparisons or by the test for linear trends between column means and column number) and the calculation of regression lines ($y = a + bx$) were performed according to conventional procedures. *P* values < 0.05 were taken to indicate statistical significance.

RESULTS

Sympathomimetic Effects in Perfused Rabbit Heart

In spontaneously beating rabbit hearts, both MIBG and tyramine elicited increases in heart rate. However, MIBG

was much less effective than tyramine (Fig. 1A). When Hill's equation was fitted to the results by nonlinear regression analysis, the maximum response obtained by extrapolation was much less pronounced for MIBG (59 ± 24 bpm) than for tyramine (156 ± 14 bpm; $P < 0.01$). The positive-chronotropic effects of 10 μmol tyramine and MIBG observed at the end of the dose-response experiments of Figure 1A did not differ from those observed in hearts in which 10 μmol tyramine or MIBG was the sole dose (Fig. 1B). Figure 1B also shows that the heart rate responses to tyramine and MIBG were markedly reduced by the uptake$_1$ blocker desipramine (2 μmol/L).

MIBG and tyramine also elicited a dose-dependent release of norepinephrine (Fig. 2A). But again, as for the heart rate response, the increase in norepinephrine overflow evoked by MIBG was much less pronounced than that evoked by tyramine. The calculated maximum norepinephrine overflow induced by MIBG and tyramine was 35 ± 8 and 218 ± 33 pmol/g ($P < 0.01$), respectively. The overflow response to both drugs was highly susceptible to inhibition by desipramine (Fig. 2B). Qualitatively similar results were obtained for the drug-induced overflow of dopamine (Figs. 2C and D). Figure 2 also shows that 0.3 μmol tyramine and 10 μmol MIBG were about equally effective not only in releasing norepineprhine but also in releasing dopamine. Hence, the difference in the abilities of MIBG and tyramine to release dopamine appeared to be quantitative and not qualitative. The ratio of dopamine overflow to norepinephrine overflow induced by tyramine increased with increasing tyramine dose (0.037 ± 0.015 at 0.3 μmol and 0.134 ± 0.011 at 10 μmol tyramine [n = 7 each; $P < 0.01$]). This kind of analysis was not possible with MIBG, because the increase in dopamine overflow observed in response to MIBG was low and inconsistent.

MIBG and tyramine differed not only with regard to the magnitude of their sympathomimetic effects but also as to the time course of their effects. As far as the heart rate responses to 0.03, 0.1, 0.3, 1, 3 and 10 μmol MIBG or tyramine are concerned (n = 7 each), the time to peak increase in heart rate was 0.2 ± 0.1, 0.4 ± 0.1, 0.6 ± 0.1, 1.9 ±Ungeträgerte Radiosynthese von meta-[^{123}I]- 0.4, 3.6 ± 0.6 and 8.6 ± 1.0 min, respectively, after administration of MIBG and 0.3 ± 0.1, 0.4 ± 0.1, 0.4 ± 0.1, 0.5 ± 0.1, 0.8 ± 0.1 and 1.5 ± 0.2 min, respectively, after administration of tyramine; the corresponding values for the time required to reach baseline heart rate again were 1.4 ± 0.6, 1.2 ± 0.4, 9.2 ± 3.1, 17.1 ± 3.5, >35 and >45 min, respectively, after administration of MIBG and 1.0 ± 0.1, 1.0 ± 0.2, 3.5 ± 0.8, 6.9 ± 1.7, 12.4 ± 0.5 and 18.0 ± 1.5 min, respectively, after administration of tyramine. This is why the preinjection heart rate before the last dose in the dose-response experiments shown in Figure 1A was higher than the baseline heart rate in hearts exposed to MIBG (185 ± 14 versus 156 ± 10 bpm; $P < 0.01$) (Table 1) but did not differ from baseline heart rate in hearts exposed to tyramine (148 ± 9 versus 156 ± 9 bpm).

In other experiments, the responses to 10 μmol MIBG and tyramine were followed for 60 min (Fig. 3). The increase in heart rate lasted more than 60 min after MIBG and approximately 20 min after tyramine injection (Fig. 3A). Accordingly, the norepinephrine overflow induced by MIBG and tyramine declined with half-lives of 57.8 ± 13.4 and 2.2 ± 0.1 min ($P < 0.01$), respectively (Fig. 3B). From the results shown in Figure 3B, the total norepinephrine overflow induced by 10 μmol MIBG (i.e., the area under the curve from 0 min to infinity) was calculated to be 79 ± 19 pmol/g, whereas the corresponding value for the total overflow evoked by tyramine was 259 ± 44 pmol/g ($P <$

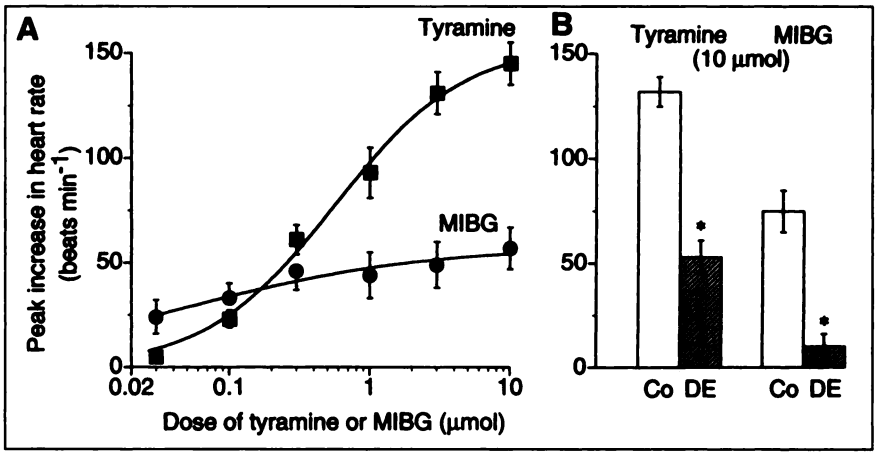

FIGURE 1. Peak increases in heart rate above baseline observed in response to series of consecutively administered MIBG or tyramine doses increasing from 0.03 to 10 μmol (A) and in response to single MIBG or tyramine dose of 10 μmol in absence (Co) or presence (DE) of 2 μmol/L desipramine (B). Isolated, spontaneously beating rabbit hearts perfused with Tyrode's solution (Langendorff technique, 37°C, 26 mL/min). MIBG and tyramine were given as bolus injections (100 μL) into aortic cannula. Baseline heart rate was 164 ± 4 bpm (n = 39). Shown are mean ± SEM of 7 (A), 8 (B; Co), 4 (B; DE, tyramine) and 5 (B; DE, MIBG) observations. Fitting Hill's equation to mean group results gave these parameters (see Materials and Methods): E_{max} 59 (MIBG) and 156 (tyramine) bpm; ED_{50} 0.1 and 0.6 μmol/L; nH 0.5 and 0.9. Dose-response curves (A) were drawn to fit these parameters. *$P <$ 0.01 for effect of desipramine.

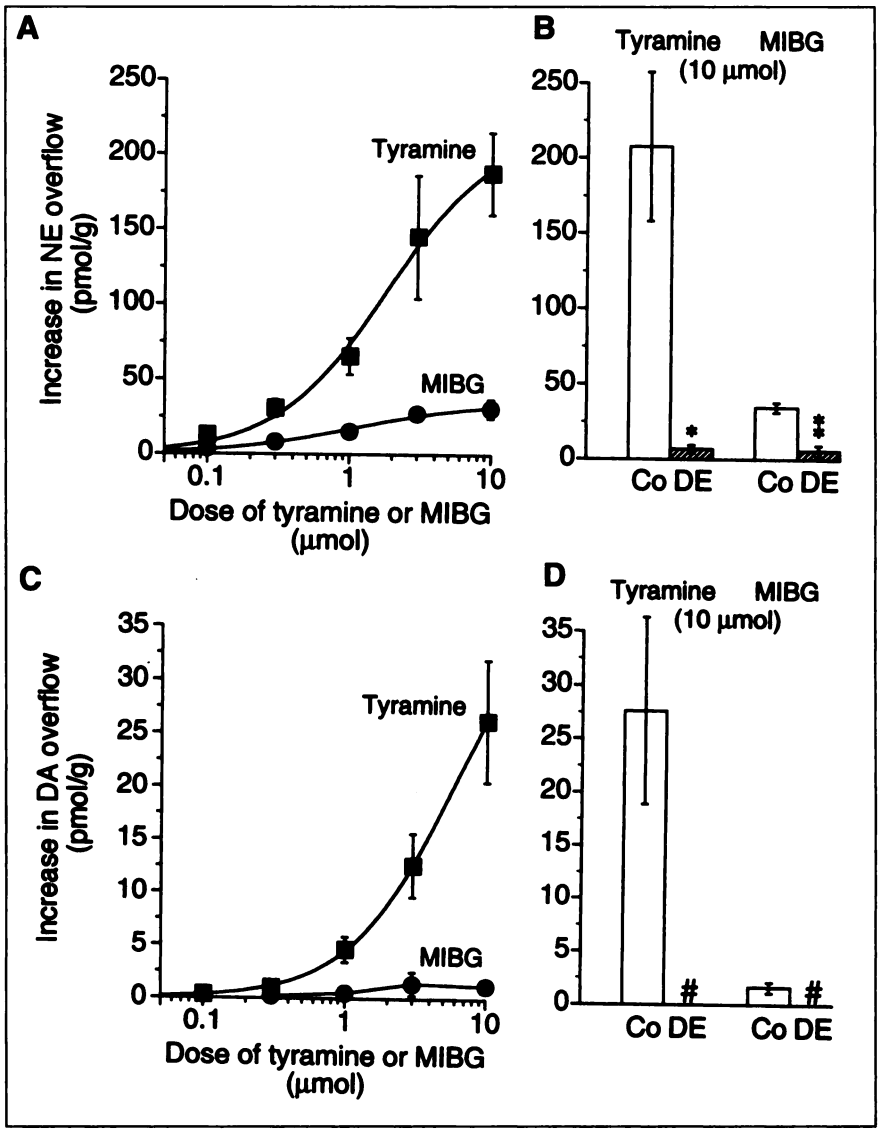

FIGURE 2. Total increases in norepinephrine (NE) (A and B) and dopamine (DA) (C and D) overflow observed in response to consecutively administered MIBG or tyramine doses increasing from 0.1 to 10 μmol (A and C) and in response to single MIBG or tyramine dose of 10 μmol in absence (Co) or presence (DE) of 2 μmol/L desipramine (B and D). Same experiments as in Figure 1. Baseline (i.e., spontaneous) NE and DA overflow was 0.50 ± 0.11 and 0.08 ± 0.02 pmol/g/min (n = 39 each), respectively. Shown are mean ± SEM of 7 (A and C), 8 (B and D; Co), 4 (B and D; DE, tyramine) and 5 (B and D; DE, MIBG) observations. Fitting Hill's equation to mean group results gave these parameters (see Materials and Methods): A, E_{max} 35 (MIBG) and 218 (tyramine) pmol/g; ED_{50} 1.1 and 1.8 μmol/L; nH 1.0 and 1.1.; C, E_{max} 40 (tyramine) pmol/g; ED_{50} 5.7 μmol/L; nH 1.2. Dose-response curves (A and C) were drawn to fit these parameters. $^*P < 0.05$ and $^{**}P < 0.01$ for effect of desipramine on NE overflow. # = No detectable increase in DA overflow in presence of desipramine.

0.01). As far as the overflow response to tyramine is concerned, this value was similar to those given in Figures 2A and B. However, the overflow responses to 10 μmol MIBG shown in Figures 2A and B were smaller than the calculated total overflow given above.

Cardiodepressant Effects of MIBG

MIBG also acted as a cardiodepressant: A dose of 10 μmol markedly decreased heart rate and, to an even greater extent, LVPP (Fig. 4). These responses to MIBG were brief in onset, dose dependent and short lasting and occurred at MIBG doses ≥0.3 μmol (Table 1). A 61%–88% decrease in LVPP was also observed after injection of 10 μmol MIBG in two hearts paced at 5 Hz.

Injections of 100 μL deionized water or saline (used as vehicle solutions) did not alter heart rate or LVPP. However, the injection of 100 μL of 100 mmol/L sodium acetate (i.e., the amount of acetate present in 10 μmol MIBG acetate) produced some decrease in heart rate and LVPP (Table 2). Nevertheless, Table 2 also shows that the cardiodepressant effect of 10 μmol MIBG acetate was clearly more pronounced than that of 10 μmol sodium acetate. Neither the injection of deionized water or saline nor the injection of 10 μmol sodium acetate caused any increases in norepinephrine or dopamine overflow (data not shown).

In hearts exposed to 2 μmol/L desipramine, the decrease in heart rate caused by 10 μmol MIBG was more pronounced and lasted much longer than in hearts not exposed to desipramine (Table 2). The MIBG-induced decrease in LVPP, on the other hand, was attenuated in the presence of desipramine (Table 2). Desipramine, per se, had no effect on heart rate, but reduced LVPP by approximately 40% (Table 2).

Uptake by SK-N-SH Cells

SK-N-SH cells were used to compare MIBG with tyramine and norepinephrine as substrates of $uptake_1$. The nisoxetine-sensitive uptake by these cells was taken to reflect membrane transport mediated by $uptake_1$. The kinetic analysis of the saturation of $uptake_1$ by these substrates showed that MIBG, tyramine and norepinephrine (all in

TABLE 1
Dose-Dependent Cardiodepressant Effects of MIBG in Perfused, Isolated, Spontaneously Beating Rabbit Heart

MIBG dose (μmol)	Preinjection HR (bpm)	Decrease in HR (%)	Time to minimum HR (s)	Time to preinjection HR (s)	Preinjection LVPP (mm Hg)	Decrease in LVPP (%)	Time to minimum LVPP (s)	Time to preinjection LVPP (s)
0.3	162 ± 14	7 ± 2	2.6 ± 0.7	7 ± 2	76 ± 6	7 ± 2	4.7 ± 1.1	11 ± 3
1	166 ± 15	6 ± 1	3.5 ± 0.9	11 ± 4	76 ± 7	15 ± 3	5.7 ± 0.7	14 ± 1
3	172 ± 15	9 ± 2	7.7 ± 1.2	30 ± 8	73 ± 7	32 ± 4	4.9 ± 0.4	39 ± 14
10	185 ± 14*	19 ± 4*	18.6 ± 3.5*	115 ± 13*	69 ± 7	64 ± 3*	5.8 ± 0.7	95 ± 28*

*$P < 0.01$ for global difference and linear trend between four dose groups, indicating dose-dependent effect of MIBG on both HR and LVPP (analysis of variance for repeated measures).

Given are mean ± SEM of seven observations each. Bolus injections (100 μL) containing 0.3–10 μmol metaiodobenzylguanidine (MIBG) were given consecutively (at intervals of 20–35 min) into aortic cannula of seven perfused rabbit hearts. Heart rate (HR) and left ventricular pulse pressure (LVPP) were monitored before (preinjection) and after each MIBG dose.

labeled form) had similar K_m and V_{max} values (Table 3). The only difference found in these experiments was that the k value for the nisoxetine-resistant (nonsaturable) component of uptake was relatively high for MIBG and tyramine compared with norepinephrine (Table 3). The k value equals the slope of the regression line relating the 1-min uptake in the presence of nisoxetine (10 μmol/L) to the substrate concentration and is probably related to the lipophilicity of the substrates.

Inhibition of Vesicular Uptake

The affinity of MIBG to the vesicular monoamine transporter was studied in membrane vesicles derived from bovine chromaffin granules. A 70%/30% mixture of epinephrine/norepinephrine (see Materials and Methods) was used as substrate, and initial rates of uptake were measured at substrate concentrations ranging from 13 to 108 μmol/L both in the absence and presence of various concentrations of MIBG. As shown in Figure 5, MIBG reduced the vesicular uptake of ^{3}H-catecholamines in a concentration-dependent manner. There was a parallel shift of the inhibition curve to the right when the substrate concentration was increased, thus suggesting that the inhibition of uptake produced by MIBG was competitive in nature. This was confirmed by further kinetic analysis of the results of Figure 5. The slope of the double-reciprocal plot of the uptake rate (1/v) versus the ^{3}H-catecholamine concentration (1/S) increased with increasing MIBG concentration, whereas the ordinate intercept of this plot remained unchanged. Moreover, the replot of the slope of the 1/v versus 1/S plot against the concentration of MIBG revealed a straight line with a coefficient of determination (r^2) of 0.998. IC_{50} values for MIBG obtained from the results shown in Figure 5 were transformed to K_i values (n = 19); the mean K_i was 20 (95% confidence limits: 17 and 23) μmol/L.

The K_i of tyramine for inhibition of the vesicular monoamine transporter determined previously under identical experimental conditions (*23*) was 2.5 (1.8 and 3.6) μmol/L. Hence, tyramine is eight times more potent than MIBG in inhibiting the vesicular uptake of catecholamines.

DISCUSSION

This study deals with the sympathomimetic action of MIBG in the isolated perfused rabbit heart. Because it was clear right from the beginning that MIBG would mainly act indirectly, the prototype indirectly acting sympathomimetic amine tyramine was included in this study. The results indicate that MIBG was indeed tyramine-like: The dose-dependent increase in heart rate induced by MIBG went hand in hand with a dose-dependent increase in norepinephrine overflow, with both effects being highly susceptible to inhibition by the uptake$_1$ blocker desipramine.

Although tyramine-like, MIBG differed from tyramine in two aspects. First, the duration of sympathomimetic action (at doses ≥1 μmol) was longer for MIBG than for tyramine and, second, MIBG was much less effective in producing sympathomimetic effects than tyramine. The long duration of action of MIBG is readily explained by the fact that MIBG, unlike tyramine, is not a substrate for monoamine oxidase. Therefore, MIBG is likely to disappear from the axoplasm of noradrenergic neurons much more slowly than tyramine. The relatively low efficacy of MIBG could be a consequence of the phenomenon of tachyphylaxis if the degree of tachyphylaxis to MIBG's action were more pronounced than that to tyramine's action. In this experimental setting, however, tachyphylaxis to either MIBG or tyramine did not develop. This was substantiated by the finding that the responses to the 10-μmol dose of either drug observed at the end of the dose-response experiments did not differ from those observed after administration of the 10-μmol dose to begin with.

The reason for MIBG being much less effective than tyramine may also reside in the complex mechanism by which indirectly acting sympathomimetic amines release norepinephrine from postganglionic sympathetic nerves. All such agents are substrates for uptake$_1$ and competitive inhibitors of the vesicular monoamine transporter; their effectiveness as norepinephrine releasers depends, above all, on the rate at which they are transported into the neuron as well as the potency with which they block the vesicular

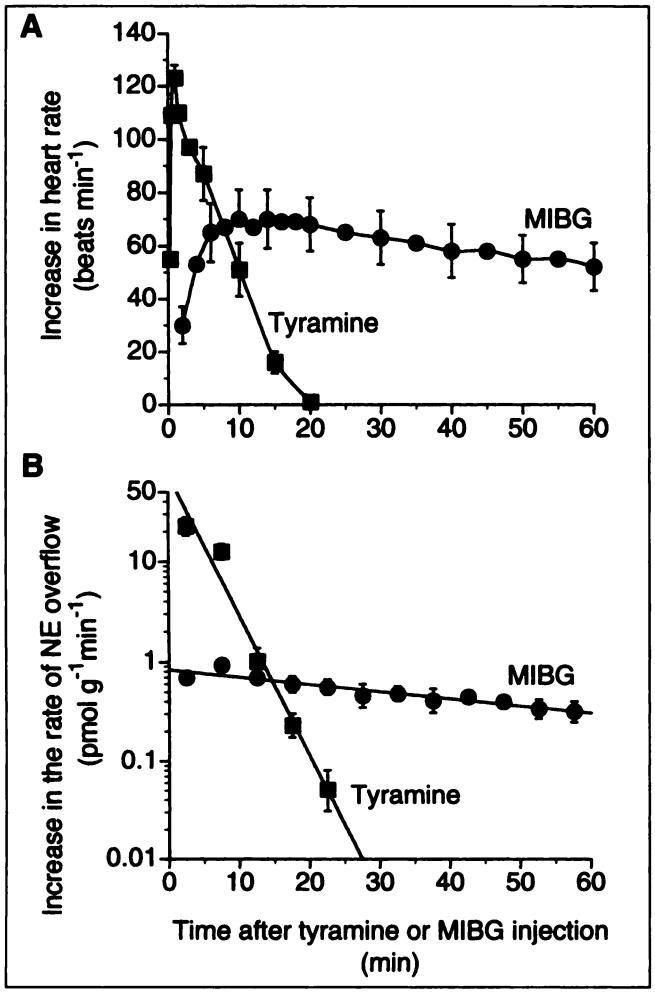

FIGURE 3. Time course of increase in heart rate (A) and norepinephrine (NE) overflow (B) elicited by bolus injection of 10 μmol MIBG or tyramine. Isolated, spontaneously beating rabbit hearts perfused with Tyrode's solution (37°C, 26 mL/min). Baseline heart rate was 165 ± 7 bpm and spontaneous NE overflow 0.36 ± 0.07 pmol/g/min (n = 16 each). Shown are arithmetic (A) or geometric (B) mean ± SEM of eight observations each. Regression lines (y = a + bx) shown in (B) were drawn to fit these equations: log y = 1.864 − 0.141X(tyramine) and log y = −0.0793 − 0.00726X(MIBG).

reuptake of norepinephrine that normally leaks out of the transmitter storage vesicles at a rather high rate (*24,25*). Synaptic vesicles function like a "pump and leak system" (*21*) and store norepinephrine by generating a dynamic equilibrium between vesicular uptake (active inward pump) and diffusion out of the vesicles (passive outward leak). Indirectly acting agents disarrange this dynamic equilibrium: They mobilize vesicularly stored norepinephrine by competing with norepinephrine for vesicular reuptake and induce a carrier-mediated transport of axoplasmic norepinephrine out of the neuron. This type of substrate-induced norepinephrine release is part of a phenomenon called "facilitated exchange diffusion," in which the neuronal norepinephrine transporter responsible for uptake$_1$ couples the inward transport of indirectly acting amines to the outward transport of axoplasmic norepinephrine (*25*).

With these considerations in mind, we compared MIBG and tyramine as substrates of uptake$_1$ in SK-N-SH cells and as inhibitors of the vesicular uptake process in membrane vesicles derived from bovine adrenomedullary chromaffin granules. SK-N-SH cells are known to express the uptake$_1$ transporter (*26,27*). The results obtained in these cells show that the saturation of uptake$_1$ by MIBG and tyramine is characterized by similar K_m and V_{max} values, indicating that, at any given substrate concentration, rates of neuronal uptake are similar for the two compounds. Hence, our finding that MIBG was a poor norepinephrine releaser compared with tyramine cannot be explained by differences between the rates of neuronal uptake of these two agents. On the other hand, as far as the inhibition of the vesicular monoamine transporter is concerned, MIBG was eight times less potent than tyramine. This observation explains why MIBG was less effective than tyramine in releasing norepinephrine, because the ability of MIBG to mobilize stored norepinephrine by inhibiting the vesicular reuptake of the transmitter is likely to be much less pronounced than that of

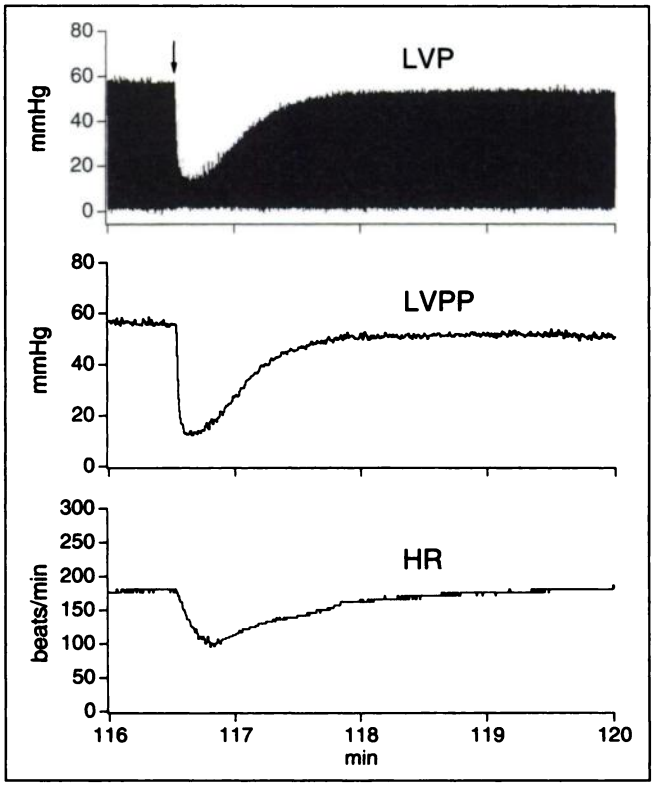

FIGURE 4. Representative tracings show transient cardiodepressant effects of MIBG in isolated, perfused, spontaneously beating rabbit heart. Hearts were perfused with Tyrode's solution (37°C, 26 mL/min), and left ventricular pressure (LVP), left ventricular pulse pressure (LVPP) and heart rate (HR) were recorded from saline-filled latex balloon inserted into left ventricle and Isotec pressure transducer connected to computer-assisted signal processing system. Arrow indicates point in time at which bolus of 10 μmol MIBG (100 μL) was injected into aortic cannula.

TABLE 2
Effect of Desipramine (DE) on Cardiodepressant Effect of 10 μmol MIBG in Perfused, Isolated, Spontaneously Beating Rabbit Heart

MIBG dose (μmol)	DE (μmol/L)	n	Baseline HR (bpm)	Decrease in HR (%)	Time to minimum HR (s)	Time to baseline HR (s)	Baseline LVPP (mm Hg)	Decrease in LVPP (%)	Time to minimum LVPP (s)	Time to baseline LVPP (s)
0	0	6	157 ± 7	6 ± 2	9.2 ± 1.9	33 ± 8	75 ± 5	14 ± 3	3.7 ± 0.2	13 ± 1
10	0	9	173 ± 11	11 ± 1*	12.3 ± 2.8	58 ± 15	68 ± 4	80 ± 2†	5.4 ± 0.4	152 ± 43*
10	2	5	168 ± 12	21 ± 1‡	21.6 ± 2.4§	242 ± 33‡	41 ± 7‡	60 ± 2‡	10.0 ± 1.9‡	115 ± 38

*$P < 0.05$ and †$P < 0.01$ when hearts not exposed to DE and injected with MIBG were compared with those injected with vehicle.

‡$P < 0.01$ and §$P < 0.05$ when hearts injected with MIBG and exposed to DE were compared with those not exposed to DE (analysis of variance followed by Bonferroni test for multiple comparisons).

Given are mean ± SEM of n observations. A 100-μL bolus containing 10 μmol sodium acetate (0 = vehicle) or 10 μmol metaiodobenzylguanidine (MIBG) acetate was injected into aortic cannula of perfused rabbit hearts. Heart rate (HR) and left ventricular pulse pressure (LVPP) were monitored before (baseline) and after vehicle or MIBG administration. In some experiments, 2 μmol/L DE was present in medium perfusing the hearts.

tyramine. In this context it must be emphasized that there are two isoforms of the vesicular monoamine transporter (VMAT1 and VMAT2) and that the main isoform operative in bovine chromaffin granules (VMAT2) is also expressed in synaptic vesicles of postganglionic sympathetic neurons (*28,29*).

The doses of MIBG used in this study cannot easily be extrapolated to give MIBG concentrations in the medium perfusing the hearts. Nevertheless, sympathomimetic effects of MIBG such as increases in heart rate and blood pressure have not been reported as common findings in the clinical setting. In other words, the plasma concentrations observed after intravenous administration of therapeutic doses of ^{131}I-MIBG (MIBG mass 25–35 μmol; plasma concentration ≤0.1 μmol/L [*2,30*]) must be less than sympathomimetically effective MIBG concentrations. Even MIBG doses of 255–290 μmol, which produced plasma concentrations of approximately 1 μmol/L when given by intravenous infusion within 3 h, were not reported to be associated with clear sympathomimetic effects (*31*). This is in agreement with preliminary

TABLE 3
Saturation Constants (K_m and V_{max}) for Uptake$_1$ and Rate Constant (k) for Nonmediated (Nonsaturable) Uptake of Labeled MIBG, Tyramine and Norepinephrine by SK-N-SH Cells

Amine	K_m (μmol/L)	V_{max} (pmol/mg protein/min)	k (pmol/mg protein/min/ μmol/L)
^{125}I-MIBG	1.6 ± 0.8	43.4 ± 10.1	10.2 ± 1.1
^{14}C-tyramine	1.7 ± 0.5	36.6 ± 2.9	9.9 ± 0.7
^{3}H-norepinephrine	1.0 ± 0.2	35.6 ± 3.7	3.2 ± 0.7*

*$P < 0.01$ compared with ^{125}I-metaiodobenzylguanidine (MIBG) and ^{14}C-tyramine (analysis of variance followed by Bonferroni test for multiple comparisons).

Given are mean ± SEM of three experiments carried out in duplicate. The 1-min cellular uptake of labeled compounds (at concentrations ranging from 0.03 to 10 μmol/L) was determined in absence (total uptake) and presence (nonmediated uptake) of 10 μmol/L nisoxetine (a selective uptake$_1$ blocker), and saturable uptake mediated by uptake$_1$ was calculated from difference between total and nonmediated uptake.

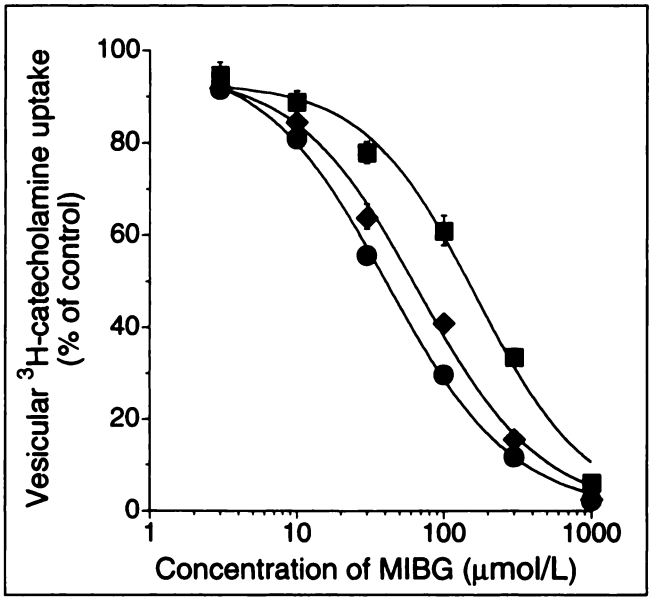

FIGURE 5. Inhibition by MIBG of vesicular ^{3}H-catecholamine uptake. Membrane vesicles obtained from bovine chromaffin granules were exposed to 12.7 ± 0.8 (●), 30.9±0.7 (◆) and 108.4 ± 6.9 (■) μmol/L of ^{3}H-catecholamine mixture (see Materials and Methods) and initial rates of reserpine-sensitive ^{3}H-catecholamine uptake were measured both in absence and presence of MIBG (3–1000 μmol/L). Control rates of uptake observed at substrate concentrations of 13, 31 and 108 μmol/L were 3.7 ± 0.5 (n = 6), 5.5 ± 0.3 (n = 7) and 6.6 ± 0.3 (n = 6) nmol/mg protein/ min, respectively. Shown are mean ± SEM of six (●), seven (◆) and six (■) observations. When Hill's equation was fitted to mean group data, the following parameters were obtained (see Materials and Methods): IC_{50} (from left to right) = 41.6, 67.7 and 166.4 μmol/L; I_{max} = 98.3%, 95.3% and 92.9%; nH = −1.02, −1.04 and −1.15. Concentration-effect curves shown in graph were drawn to fit these parameters.

findings that, in the perfused rabbit heart, 1 μmol/L MIBG was a threshold concentration for the positive chronotropic effect of the drug (Bossle, Wölfel and Graefe; unpublished observations).

Female, athymic, nude mice injected intravenously with 10 μmol MIBG died immediately after the injection (*32*). This finding might have been due to the indirect sympathomimetic effects of MIBG. Alternatively, the mice may have died as a result of the cardiodepressant action of MIBG observed in this study. This action may be related to MIBG acting as a reversible inhibitor of the mitochondrial respiration (*33,34*). The cardiodepressant effects manifested themselves in initial short-lived decreases in heart rate and LVPP. Only when the heart rate is held constant, the value of LVPP reflects the inotropic state of the left ventricle. Because the marked decrease in LVPP was observed not only in spontaneously beating but in paced hearts, it can be concluded that MIBG compromises both the pacemaker automaticity and the myocardial contractility. The transient nature of the cardiodepressant effects of MIBG was obviously a consequence of the way MIBG was administered. Bolus injections into the aortic cannula do not produce sustained MIBG concentrations in the perfusion medium and, hence, will not bring about sustained cardiodepressant effects.

Because MIBG's cardiodepressant and sympathomimetic actions were observed in the same dose range, it can be inferred that the negative chronotropic and inotropic effects of the drug are unlikely to become apparent in the clinical setting, just as the occurrence of the sympathomimetic effects of MIBG is uncommon in patients given diagnostic or therapeutic doses of radioiodinated MIBG (see above). One may even envisage the possibility that the sympathomimetic effects and initial cardiodepressant of MIBG mask each other. As far as the negative chronotropic action of MIBG is concerned, this possibility was verified in this study by the finding that desipramine, which inhibited the drug-induced norepinephrine release, enhanced the initial heart rate decrease caused by MIBG and greatly prolonged the duration of this drug action.

CONCLUSION

In spontaneously beating, isolated, perfused rabbit hearts, MIBG was tyramine-like in that it behaved as an indirectly acting sympathomimetic agent. It was much less effective than tyramine in releasing endogenous norepinephrine, because its ability to mobilize norepinephrine from the transmitter storage vesicles was much less pronounced than that of tyramine. The duration of action of high doses of MIBG was much longer than that of tyramine. This is a consequence of the fact that MIBG is cleared from the axoplasm of noradrenergic neurons much more slowly than tyramine, because, unlike tyramine, MIBG is not metabolized by monoamine oxidase and because MIBG is taken up by synaptic vesicles much less avidly than tyramine. In contrast to tyramine, MIBG also had cardiodepressant effects that were observed before the occurrence of the drug's sympathomimetic effects. Neither the sympathomimetic nor the cardiodepressant effects of MIBG are likely to come into play in patients given diagnostic or therapeutic doses of radioiodinated MIBG not exceeding 4 μmol/kg.

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REFERENCES

1. Shapiro B, Gross MD. Radiochemistry, biochemistry, and kinetics of ^{131}I-metaiodobenzylguanidine (MIBG) and ^{123}I-MIBG: clinical implications of the use of ^{123}I-MIBG. *Med Pediatr Oncol.* 1987;15:170–177.
2. Wafelman AR, Hoefnagel CA, Maes RAA, Beijnen JH. Radioiodinated metaiodobenzylguanidine: a review of its biodistribution and pharmacokinetics, drug interactions, cytotoxicity and dosimetry. *Eur J Nucl Med.* 1994;21:545–559.
3. Farahati J, Mueller SP, Coenen HH, Reiners C. Scintigraphy of neuroblastoma with radioiodinated m-iodobenzylguanidine. In: Treves ST, ed. *Paediatric Nuclear Medicine*, 2nd ed. New York, NY: Springer; 1995:528–545.
4. Iversen LL. *The Uptake and Storage of Noradrenaline in Sympathetic Nerves.* Cambridge, UK: Cambridge University Press; 1967.
5. Wieland DM, Brown LE, Rogers WL, et al. Myocardial imaging with a radioiodinated norepinephrine storage analog. *J Nucl Med.* 1981;22:22–31.
6. Nakajo M, Shimabukuro K, Yoshimura H, et al. Iodine-131 meta-iodobenzylguanidine intra- and extravesicular accumulation in the rat heart. *J Nucl Med.* 1986;27:84–89.
7. Jaques S, Tobes MC, Sisson JC, Baker JA, Wieland DM. Comparison of the sodium dependency of uptake of meta-iodobenzylguanidine and norepinephrine into cultured bovine adrenomedullary cells. *Mol Pharmacol.* 1984;26:539–546.
8. Tobes MC, Jaques S, Wieland DM, Sisson JC. Effect of uptake-one inhibitors on the uptake of norepinephrine and metaiodobenzylguanidine. *J Nucl Med.* 1985;26:897–907.
9. Buck J, Bruchelt G, Girgert R, Treuner J, Niethammer D. Specific uptake of m-[^{125}I] iodobenzylguanidine in the human neuroblastoma cell line SK-N-SH1. *Cancer Res.* 1985;45:6366–6370.
10. Smets LA, Janssen M, Rutgers M, Ritzen K, Buitenhuis C. Pharmacokinetics and intracellular distribution of the tumor-targeted radiopharmaceutical m-iodobenzylguanidine in SK-N-SH neuroblastoma and PC-12 pheochromocytoma cells. *Int J Cancer.* 1991;48:609–615.
11. Gasnier B, Roisin M-P, Scherman D, Coornaert S, Desplanches G, Henry J-P. Uptake of meta-iodobenzylguanidine by bovine chromaffin granule membranes. *Mol Pharmacol.* 1986;29:275–280.
12. DeGrado TR, Zalutsky MR, Vaidyanathan G. Uptake mechanisms of meta-[^{123}I]iodobenzylguanidine in isolated rat heart. *Nucl Med Biol.* 1995;22:1–12.
13. Slosman DO, Davidson D, Brill AB, Alderson PO. ^{131}I-meta-iodobenzylguanidine uptake in the isolated rat lung: a potential marker of endothelial cell function. *Eur J Nucl Med.* 1988;13:543–547.
14. Rutgers M, Tytgat GAM, Verwijs-Janssen M, Buitenhuis C, Voute PA, Smets LA. Uptake of the neuron-blocking agent meta-iodobenzylguanidine and serotonin by human platelets and neuro-adrenergic tumour cells. *Int J Cancer.* 1993;54:290–295.
15. Wieland DM, Brown LE, Tobes MC, et al. Imaging the primate adrenal medulla with [^{123}I]- and [^{131}I]-meta-iodobenzylguanidine: concise communication. *J Nucl Med.* 1981;22:358–364.
16. Mangner TJ, Tobes MC, Wieland DM, Sisson JC, Shapiro B. Metabolism of ^{131}I-meta-iodobenzylguanidine in patients with metastatic pheochromocytoma. *J Nucl Med.* 1986;27:37–44.
17. Wieland DM, Wu J-I, Brown LE, Mangner TJ, Swanson DP, Beierwaltes WH. Radiolabeled adrenergic neuron-blocking agents: adrenomedullary imaging with [^{131}I]-iodobenzylguanidine. *J Nucl Med.* 1980;21:349–353.
18. Dutschka K, Coenen HH. No carrier added radiosynthesis of meta-[^{123}I]-iodobenzylguanidine and 3β-(4-[^{123}I]-iodophenyl)tropane-2β-carboxylic acid methylester by nonisotopic, Cu(I)-assisted halogen exchange [in German]. *Nucl Med.* 1994;33:A11.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RF. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193:265–275.
20. Kobold G, Langer R, Burger A. Does the carrier of chromaffin granules transport the protonated or the uncharged species of catecholamines? *Naunyn Schmiedebergs Arch Pharmacol.* 1985;331:209–219.

21. Michalke W, Langer R, Burger A. Ghosts of chromaffin granules accumulate biogenic amines according to a "pump and leak system" without contribution of carrier-mediated efflux. *Naunyn Schmiedebergs Arch Pharmacol.* 1990;342:312–322.
22. Cheng Y-C, Prusoff WH. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol.* 1973;22:3099–3108.
23. Fischer P, Burger A, Graefe K-H, Trendelenburg U. The effect of (±)-dobutamine on adrenergic nerve endings. *Naunyn Schmiedebergs Arch Pharmacol.* 1989;339: 79–84.
24. Langeloh A, Trendelenburg U. The mechanism of the ^{3}H-noradrenaline releasing effect of various substrates of uptake$_1$: role of monoamine oxidase and of vesicularly stored ^{3}H-noradrenaline. *Naunyn Schmiedebergs Arch Pharmacol.* 1987;336:611–620.
25. Bönisch H, Trendelenburg U. The mechanism of action of indirectly acting sympathomometic amines. In: Trendelenburg U, Weiner N, eds. *Catecholamines II.* Handbook of Experimental Pharmacology, vol. 90/I. Berlin, Germany: Springer; 1988:247–277.
26. Richards ML, Sadee W. Human neuroblastoma cell lines as models of catechol uptake. *Brain Res.* 1986;384:132–137.
27. Bryan-Lluka LJ, Bönisch H. Lanthanides inhibit the human noradrenaline, 5-hydroxytryptamine and dopamine transporters. *Naunyn Schmiedebergs Arch Pharmacol.* 1997;355:699–706.
28. Erickson JD, Schäfer M K-H, Bonner TI, Eiden LE, Weihe E. Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proc Natl Acad Sci USA.* 1996;93:5166–5171.
29. Henry JP, Sagné C, Botton D, Isambert MF, Grasnier B. Molecular pharmacology of the vesicular monoamiune transporter. In: Goldstein DS, Eisenhofer G, McCarty R, eds. *Catecholamines: Bridging Basic Science with Clinical Medicine.* London, UK: Academic Press; 1998:236–239.
30. Smets LA, Loesberg C, Janssen M, Metwally EA, Huiskamp R. Active uptake and extravesicular storage of m-iodobenzylguanidine in human neuroblastoma SK-N-SH cells. *Cancer Res.* 1989;49:2941–2944.
31. Wafelman AR, Konings MCP, Rosing H, et al. High-performance liquid chromatographic determination of metaiodobenzylguanidine in whole blood and plasma of cancer patients. *J Pharm Biomed Anal.* 1994;12:1173–1179.
32. Farahati J, Coenen HH, Dutschka K, et al. Effect of specific activity on neuroblastoma uptake of I-123-meta-iodobenzylguanidine in nude mice xenografted with SK-N-SH cells. *Oncol Reports.* 1997;4:363–366.
33. Loesberg C, Van-Rooij H, Nooijen WJ, Meijer AJ, Smets LA. Impaired mitochondrial respiration and stimulated glycolysis by m-iodobenzylguanidine (MIBG). *Int J Cancer.* 1990;46:276–281.
34. Van den Berg J, Smets LA, Rutgers M, et al. Chemical characterization and comparative cellular effects of meta-iodobenzyl guanidine and benzyl guanidine. *Cancer Chemother Pharmacol.* 1997;40:131–137.