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Renal toxicity of the neuron-blocking and mitochondriotropic agent *m*-iodobenzylguanidine

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Abstract *meta*-Iodobenzylguanidine (MIBG) is a multipotent drug used in its radiolabeled form as a tumor-seeking radiopharmaceutical in the diagnosis and treatment of pheochromocytoma and neuroblastoma. Nonradiolabeled MIBG has also proved to be effective in the palliation of carcinoid syndromes and, on a predosing schedule, in enhancing the relative tumor uptake of a subsequent [^{131}I]-MIBG dose in tumors of neuroadrenergic origin. In addition, MIBG is under investigation as an inhibitor of mitochondrial respiration and, as such, for its use in tumor-specific acidification. In this report we describe the side effects of nonradiolabeled MIBG on kidney function in mice. High doses of MIBG (40 mg/kg) reduced renal blood perfusion as measured by ^{86}Rb distribution by 50%, which could be antagonized by the bioamine receptor blockers prazosin and cyproheptadine. MIBG also induced reversible renal damage as evidenced from a decrease in [^{51}Cr]-ethylenediaminetetraacetic acid (EDTA) clearance and from histological damage, which was most pronounced in the distal tubuli. These effects were unrelated to reduced perfusion, however, and could not be antagonized by bioamine receptor blockers, Ca^{2+}-channel blockers, or diuretics. Clearance effects of MIBG were mimicked by *N*-nitro-L-arginine methyl ester (L-NAME), a known inhibitor of nitric oxide synthase (NOS), and MIBG itself (100 μM) also inhibited NOS in vitro, suggesting that NOS inhibition by MIBG may have contributed to the observed reduction in renal clearance. The MIBG analog benzylguanidine (BG), which is equipotent in terms of mitochondrial inhibition, did not affect renal clearance, thus excluding mitochondrial inhibition as the main mechanism of MIBG-induced damage. MIBG, however, was much more cytotoxic than BG to kidney tubular cells in primary cultures. Although the renal effects of high-dose MIBG were reversible, alterations in the pharmacokinetics of concomitant medications by a temporary reduction in renal function should be taken into account in its clinical application.

Key words MIBG · Kidney · Bioamines · Mitochondrial respiration · NOS

Abbreviations *MIBG meta*-Iodobenzylguanidine · *BG* Benzylguanidine · *NO* Nitric oxide · *NOS* Nitric oxide synthase · L-*NAME N*-Nitro-L-arginine methyl ester · *PBS* Phosphate-buffered saline · *NAG N*-Acetyl-β-D-glucosaminidase · *FCS* Fetal calf serum · *PEG* Polyethylene glycol

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Introduction

meta-Iodobenzylguanidine (MIBG) is a multipotent drug that is increasingly being applied in clinical oncology. Radio-iodinated MIBG ([^{131}I]-MIBG) has primarily been developed as a derivative of the neuron-blocking agents bretylium and guanethidine for the scintigraphic detection of neuroadrenergic tissues [26]. Due to its affinity for the specific Uptake-1 mechanism of catecholamines [6, 18] and the serotonin transporter [13], [^{131}I]-MIBG is currently applied for the diagnosis and treatment of pheochromocytoma, neuroblastoma, and carcinoid tumors [3, 21].

Nonradioactive MIBG is occasionally given to neuroblastoma and carcinoid patients on a predosing schedule to improve the relative tumor uptake of a subsequent [^{131}I]-MIBG dose [22]. Furthermore, long-lasting palliative responses have been observed in about 60% of carcinoid patients following treatment with nonradiolabeled MIBG alone [21]. Because MIBG can abrogate resistance to glucocorticoid hormones [16], the drug is also under investigation as a potential reversal agent in glucocorticoid-resistant acute lymphoblastic leukemia (personal communication, G.J. Kaspers, Free University, Amsterdam, The Netherlands). A special application of MIBG that is of potential clinical interest derives from its documented potency as an inhibitor of mitochondrial respiration, leading to a compensatory increase in glycolytic flux in tissue-culture cells [9]. MIBG, supported by parenteral glucose, selectively decreases the intratumoral pH by a full pH unit below that of normal tissue [7] and has subsequently been applied to potentiate the antitumor response to camptothecin [2].

The increased actual and projected clinical use of MIBG in patients warrants studies of possible side effects associated with its use in high-dose or frequent application. In mice the drug is acutely lethal at i.p. doses over 40 mg/kg [15], and at doses below 40 mg/kg, stress-related sympathomimetic side effects have been observed, plausibly explained by the release of bioamines from their storage granules [7]. Moreover, increased toxicity of the cytostatic agent melphalan in MIBG/glucose-treated mice was suggestive of impaired renal clearance of the drug (unpublished results). In patients, transient changes in blood pressure, dizziness, and paleness have been observed following doses of up to 135 mg/m^2 ([21]; personal communication, B. Taal, The Netherlands Cancer Institute).

In the present report we describe the adverse effects of MIBG on renal function in mice. In addition to histology, renal function and perfusion were investigated as the rate of clearance of [^{51}Cr]-ethylenediaminetetraacetic acid (EDTA) and the tissue distribution of ^{86}Rb, respectively. The α_1 receptor-blocker prazosin and the 5-hydroxytryptamine (5-HT) receptor-blocker cyproheptadine were tested for possible antagonism of sympathomimetic responses. Furthermore, the possible involvement of ischemia was investigated by comparison of the effects of MIBG with those of L-NAME, a known vasoconstrictive agent, by inhibition of nitric oxide synthases (NOS) [1]. Because of its structural similarity to L-arginine (Fig. 1), MIBG was tested for its capacity to inhibit NOS. In an attempt to prevent nephrotoxicity, MIBG was combined with the Ca^{2+}-channel blockers verapamil and nifedipine, which are known to prevent ischemic damage [12]. Furthermore, we tested whether blocking of the activity of distal tubuli by the diuretics amiloride and bendroflumethiazide could antagonize renal damage. Finally, MIBG was compared with the analog benzylguanidine (BG; Fig. 1), which is almost as effective as MIBG in mitochondrial inhibition but has 5-fold lower affinity for Uptake 1 [23]. This comparison served to dissociate antimitochondrial effects from other cytotoxic actions possibly involved in MIBG-induced renal damage.

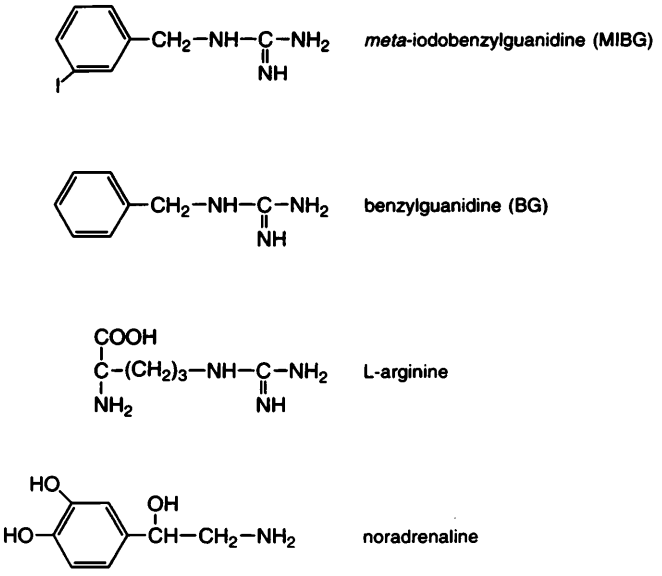

Fig. 1 Structures of *meta*-iodobenzylguanidine (*MIBG*), benzylguanidine (*BG*), L-arginine, and noradrenaline

Materials and methods

Animals and treatment protocols

Male C3H/Km mice were used at the age of 10–12 weeks. Body weights ranged between 30 and 35 g. Mice were treated by a bolus i.p. injection of 40 mg/kg MIBG, i.e., the maximal tolerated dose [15] and the dose that is effective in tumor-selective acidification [7]. In renal function studies, MIBG was also given at 30 and 50 mg/kg. The analog BG was given at 43 mg/kg, which is below the maximal tolerated dose but twice the molar dose of MIBG as based on EC_{50} concentrations for stimulated glycolysis in vitro [23] and confirmed in pilot experiments for equipotency to MIBG in raising plasma lactate levels. MIBG and BG were given with or without 1.5 or 3.0 g/kg D-glucose. All other drugs were given at concentrations reported to be effective in mice and were tested for the absence of acute toxicity following single-agent or combination administration with MIBG. Prazosin (4 mg/kg) and cyproheptadine (1 mg/kg) were given at 1 h after MIBG/glucose treatment, whereas verapamil (1 and 10 mg/kg), nifedipine (1 mg/kg), amiloride (10 mg/kg), and bendroflumethiazide (5 mg/kg) were injected at 3 h before MIBG/glucose treatment. *N*-Nitro-L-arginine methyl ester (L-NAME) was given at 100 mg/kg. All drugs were injected i.p. in a volume of 0.01 ml/g body weight, and control mice received corresponding volumes of phosphate-buffered saline (PBS). The animal experiments were carried out in accordance with protocols approved by the experimental animal welfare committee of the institute and conformed to national and European regulations for animal experimentation.

Drugs and radiolabeled compounds

MIBG was purchased from EMKA Chemie (Markgröningen, Germany). Synthesis of benzylguanidine (BG) and purity control by positive- and negative-ion fast-atom-bombardment (FAB)-mass spectrometry analysis were performed as described previously [23].

[^{51}Cr]-EDTA (specific activity 1–2 mCi/mg), [^{86}Rb]-Cl (specific activity 1–8 mCi/mg), L-[2,3,4,5-^{3}H]-arginine-HCl (specific activity > 58 Ci/mmol), and [^{3}H]-uridine (specific radioactivity 158 mCi/mg) were obtained from Amersham International (Buckinghamshire, UK). Flavine adenine dinucleotide (FAD), glutathione, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Boehringer Mannheim (Mannheim, Germany), as was (6*R*)-5,6,7,8-tetrahydro-L-biopterin from Schircks Labs (Jona, Switzerland). All other drugs were obtained from Sigma (St. Louis, USA).

MIBG and BG were dissolved in PBS at 37 °C to a concentration of 4.0 and 4.3 mg/ml, respectively. For combination treatment, glucose was added to a final concentration of 0.15 or 0.3 g/ml. Prazosin and amiloride were dissolved in sterile demineralized water to a concentration of 0.4 and 1 mg/ml, respectively. Cyproheptadine was dissolved in 4% ethanol solution to 1 mg/ml and was diluted 10-fold in PBS before use. Bendroflumethiazide was prepared in 100% ethanol to 10 mg/ml and diluted to 0.5 mg/ml in sterile demineralized water. Nifedipine was initially dissolved in 100% ethanol (10 mg/ml), diluted 5-fold in a PEG 400 solution (0.3 g/ml 30% ethanol), and, finally, diluted in PBS to 0.1 mg/ml. Verapamil and L-NAME were dissolved in PBS to 0.1, 1.0, and 10 mg/ml, respectively.

Measurement of renal function

Glomerular filtration was determined as the plasma clearance of [^{51}Cr]-EDTA [20]. In brief, mice were injected i.p. or i.v. at several time points after MIBG/glucose treatment with 10 μCi [^{51}Cr]-EDTA in 100 μl. At 30 min after injection, blood samples were taken from the retro-orbital sinus in heparinized capillary tubes and the mice were killed. Plasma [^{51}Cr]-EDTA levels were determined by gamma-counting of 20 μl plasma; for calibration, 20 μl of the injection solution (diluted 500-fold) was taken. Results are expressed as percentages of the injected dose per milliliter of plasma, and increases in residual plasma [^{51}Cr]-EDTA levels reflect a reduced renal clearance.

Vascular perfusion using [^{86}Rb]-Cl distribution

Vascular perfusion relative to the cardiac output was measured using the [^{86}Rb]-Cl extraction technique [14, 24]. In brief, mice were injected i.v. with 10 μCi [^{86}Rb]-Cl in 100 μl 0.9% NaCl. After 1 min, mice were killed by cervical dislocation. The kidney, parts of the liver, the skeletal muscle of the hind leg, and the tail, including the injection site, were removed and weighed and the radioactivity was estimated by standard gamma-counting. The total injected dose was calculated by correction of the amount of delivered radioactivity for the residual activity in the tail. If the latter exceeded 10% of the injected dose, mice were excluded from analysis. Tissue levels were expressed as percentages of the injected dose per gram of tissue.

Urine measurements

Urine was collected from mice placed individually in cages with a wire grid floor for 24 h. Beneath the floor, absorbent paper was drawn (15 cm/h). The urinary volume was calculated from the urine-spot areas as previously described [11]. For biochemical measurements in urine, mice were held individually in metabolic cages for 16 h overnight. Urinary pH was measured by a standard pH electrode (Biotrode, Hamilton, Switzerland). Samples were frozen until the estimation of glucose, lactate, and *N*-acetyl-β-D-glucosaminidase (NAG) by commercial assays obtained from Boehringer (Mannheim, Germany).

Histology

At 3–5 h after MIBG/glucose, BG/glucose, or L-NAME administration and at 3 days after MIBG/glucose treatment, mice were killed. After fixation in 4% buffered formaldehyde, tissues were processed for histology examination; 5-μm sections were stained by standard hematoxylin and eosin staining and were examined by an animal pathologist and a consulted nephrologist.

NOS activity

Recombinant human NOS I, NOS II, and NOS III were purified from a baculovirus-expression system by 2′-5′-ADP-Sepharose affinity chromatography (A. Frey and H.H.H.W. Schmidt, unpublished data). NOS activity was measured by the conversion of L-arginine to L-citrulline [4]. The enzymes were incubated for 30 min at 37 °C in a total volume of 0.1 ml buffer containing 50 m*M* triethanolamine HCl (pH 7.2), 0.5 μ*M* calmodulin, 0.5 m*M* $CaCl_2$, 5 μ*M* FAD, 5 μ*M* FMN, 250 μ*M* 3-[(3-cholamidpropyl)dimethylammonio]-2-hydroxy-1-propan-sulfonate, 5μ*M* (6*R*)-5,6,7,8-tetrahydro-L-biopterin, 1 m*M* NADPH, 7 m*M* glutathione, and 10 or 50 μ*M* L-arginine, including 0.15 μCi L-[2,3,4,5-^{3}H]-arginine and 0.1 or 1.0 m*M* MIBG or water. The reaction was stopped with 0.9 ml cold-stop buffer (20 m*M* sodium acetate, 2 m*M* EDTA, pH 5.5). Citrulline was extracted by chromatography on a 0.8-ml cation-exchange column (Dowex AG 50 W-X8 resin, Na^+ form) that had been preequilibrated with stop buffer. The combined column flow-through (1 ml) and the water eluate (2 ml) were measured for tritium radioactivity in 10 ml Rotiszint eco and by liquid scintillation counting. All measurements were done in triplicate.

Biochemical measurements

Glucose and lactate levels in plasma were determined as described elsewhere [7]. In brief, metabolic activity was blocked by the addition of sodium fluoride at 2 mg/ml and of potassium oxalate to the blood samples before the plasma was isolated and frozen. Glucose and lactate were measured spectrophotometrically by routine assays (Boehringer Mannheim, Mannheim, Germany).

Cytotoxicity of MIBG and BG to L1210 and explanted kidney cells

Mouse leukemia L1210 cells were cultured under standard conditions (37 °C, 6% CO_2) in RPMI 1640 medium (Gibco Europe, Breda, The Netherlands) supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 μg/ml), and 60 μ*M* β-mercaptoethanol. For single-cell survival experiments, cells were suspended in a semi-solid plating medium consisting of complete RPMI 1640 supplemented with 20% conditioned medium and 0.6% carboxymethyl cellulose (Fluka, Buchs, Switzerland) to a concentration of 30 cells/ml. Increasing concentrations of MIBG or BG (3–60 μ*M*) were added, and 10 ml cell suspension (300 cells) was divided over three wells of a six-well cluster plate (Costar, Cambridge, USA). Macroscopic colonies were counted after 8–9 days. Survival was calculated as a percentage of control.

Primary culture of kidney cells from C3H/Km mice was obtained by incubation of kidney fragments for 45 min at 37 °C in PBS containing collagenase (255 U/ml), hyaluronidase (320 U/ml), bovine serum albumin (4 mg/ml), and glucose (10 m*M*). Next, fragments were further digested for 30 min at 37 °C with pronase (77.5 U/ml) in Dulbecco's modified Eagle's medium (DMEM, Gibco Europe). Enzyme activity on cells was stopped by the addition of an equal volume of FCS. After centrifugation and resuspension of the cell pellet in DMEM [supplemented with 10% FCS, penicillin (100 U/ml), and streptomycin (100 μg/ml)], the typical structure of fragmented tubuli was clearly visible. Fragments and single cells were plated into 12-well cluster plates (Falcon, New Jersey, USA). After 3 days, nonadherent cells and contaminating erythrocytes were washed off and viable adherent cells were incubated with increasing concentrations of MIBG or BG (1.5–60 μ*M*) for 12 or 24 h. Subsequently, metabolic activity was measured as the [^{3}H]-uridine incorporation observed during a 2-h period of incubation with [^{3}H]-uridine at 1 μCi/ml.

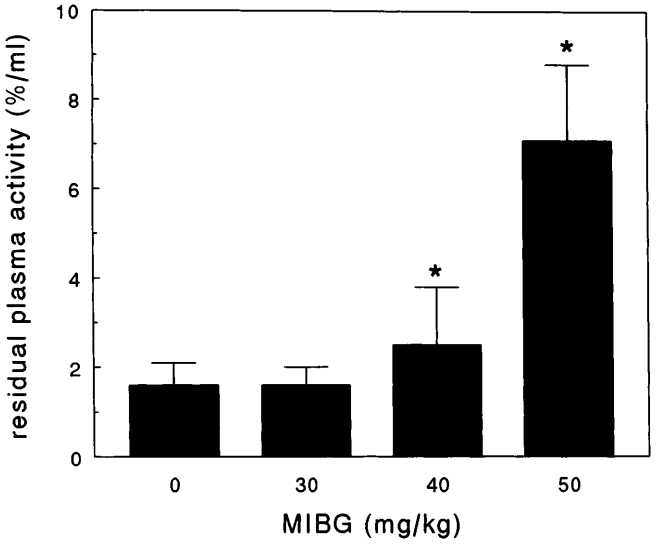

Fig. 2 Residual [^{51}Cr]-EDTA plasma activity (% of injected dose/ml plasma) as measured at 3 h after MIBG treatment of C3H/Km mice. [^{51}Cr]-EDTA was injected i.p. at 30 min before blood sampling. *Bars* represent mean values ± SD for 7–8 mice; *$P < 0.05$ versus control

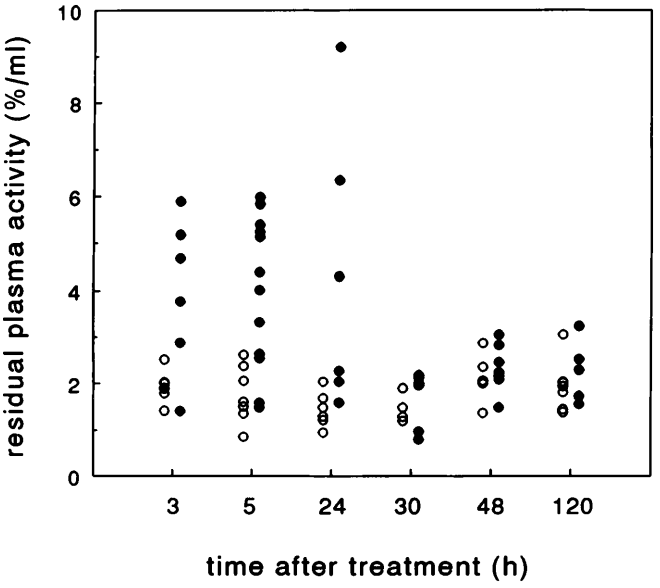

Fig. 3 Residual [^{51}Cr]-EDTA plasma activity (% of injected dose/ml plasma) as determined at different time points after PBS treatment of control animals (○) or MIBG (40 mg/kg) plus glucose (1.5 g/kg) treatment (●)

Incorporated [^{3}H]-uridine was counted and calculated as a percentage of the incorporation in untreated cells. Protein content was measured according to Lowry et al. [10].

Statistical analysis

The significance of differences in mean values was determined by Student's *t*-test. A *P* value of <0.05 was judged to be of statistical significance.

Results

Effect of MIBG and glucose on renal clearance

MIBG treatment at concentrations over 40 mg/kg caused increased residual plasma activity of [^{51}Cr]-EDTA, which was most pronounced at the toxic dose of 50 mg/kg (Fig. 2). Addition of glucose further reduced renal clearance, with residual plasma [^{51}Cr]-EDTA levels increasing from 2.6 ± 1.1% for MIBG alone (40 mg/kg) to 4.2 ± 1.0% for MIBG in combination with 1.5 g/kg glucose ($P < 0.01$). Although i.p.-injected glucose affects the pharmacokinetics of i.p.-injected [^{51}Cr]-EDTA, comparison of i.p. with i.v. injection of [^{51}Cr]-EDTA excluded that the increased values resulting from the combined treatment were due to these changed [^{51}Cr]-EDTA kinetics (not shown). In all subsequent experiments a dose of 40 mg/kg MIBG combined with 1.5 g/kg glucose was used as standard treatment and is referred to hereafter as MIBG/glucose treatment.

Impaired renal clearance is reversible and accompanied by histological damage

Reduced [^{51}Cr]-EDTA clearance was evident at 3 h after MIBG/glucose treatment and recovered within 30 h (Fig. 3). During the first 24 h after MIBG/glucose administration the urinary volume increased significantly. Also, glucose and lactate concentrations in urine increased by 5- and 15-fold, respectively, and the increases were much more extensive for MIBG/glucose than for MIBG alone (Table 1). The values correspond to the increases in plasma values recorded for glucose and lactate in this investigation (Fig. 5) and a previous study [7].

Impaired [^{51}Cr]-EDTA clearance was accompanied by histological damage. Microscope analysis revealed

Table 1 Urinary parameters of C3H/Km mice after administration of MIBG and glucose alone or in combination (*ND* Not done)

	Volume[a] in ml (*n*)	Mean pH[b] (*n*)	Lactic acid[b] in m*M* (*n*)	Glucose[b] in m*M* (*n*)	NAG[b] in U/I (*n*)
PBS	1.1 ± 0.5 (12)	6.13 ± 0.07 (4)	0.7 ± 0.3 (4)	1.8 ± 0.8 (4)	95 ± 32 (8)
Glucose 1.5 g/kg	0.7 ± 0.5 (6)	6.13 ± 0.09 (4)	1.5 ± 0.6 (4)	0.7 ± 0.2 (4)	ND
MIBG 40 mg/kg	1.3 ± 0.3 (6)	6.06 ± 0.06 (4)	11 ± 7 (4)	9 ± 5 (4)	ND
MIBG 40 mg/kg + glucose 1.5 g/kg	1.8 ± 0.6 (16)	5.9 ± 0.3 (4)	26 ± 11 (4)	47 ± 24 (4)	118 ± 40 (9)

[a] 24 h, mouse lavatory
[b] 16 h, metabolic cages

Table 2 Perfusion as measured by ^{86}Rb distribution at 3 h after treatment. [^{86}Rb]-Cl (10 μCi/mouse) was injected 1 min before the animal was killed

	Perfusion as % ^{86}Rb/g tissue (*n*)		
	Kidney	Liver	Muscle
PBS	11 ± 3 (12)	1.8 ± 0.4 (12)	3.3 ± 0.4 (12)
MIBG 40 mg/kg + glucose 1.5 g/kg	6 ± 2* (12)	1.9 ± 0.7 (12)	3.3 ± 0.6 (12)
MIBG 40 mg/kg	6 ± 1* (7)	2.0 ± 0.2 (7)	3.5 ± 0.2 (7)

*$P < 0.001$ versus control

acute degeneration and vacuolization of renal epithelial cells of the corticomedullar tubuli; dilatation, which was most prominent in the distal tubuli; and minor hypertrophy of the epithelium of Bowman's capsule. MIBG/glucose treatment did not differ from injection of MIBG alone in terms of the level of histological damage, and individual animals that displayed normal [^{51}Cr]-EDTA clearance after MIBG/glucose treatment nonetheless showed microscopic tubular damage. At 3 days after treatment, nearly all tissue damage had regenerated.

A sensitive biochemical assay of renal damage in humans is the urinary level of the epithelial protein NAG [5]. As compared with the basal levels measured in humans (0.4–5.6 U/I), the basal NAG levels determined in C3H/Km mice were very high (95 ± 32 U/I). MIBG/glucose treatment further increased NAG levels slightly, albeit significantly (Table 1; $P = 0.05$). Because of the high basal levels and considerable interanimal variation, this technique was not considered to be an adequate biochemical marker of renal damage in the present experiments.

MIBG effects on renal blood perfusion

Reduced clearance and tissue damage were accompanied by a decrease in renal perfusion as measured by [^{86}Rb]-Cl distribution (Table 2), which corresponds to the fractional distribution of the cardiac output [14]. Since perfusion was not changed in the liver or muscle, variation of cardiac output would seem less important than local hemodynamic effects in the kidney.

Comparison of MIBG with the NOS inhibitor L-NAME

To test the possibility that the nephrotoxicity of MIBG/glucose treatment was caused by local ischemia, we treated the animals with L-NAME, an inhibitor of NOS. Reduced NO production leads to a reduction in the glomerular capillary pressure via vasoconstriction of the afferent blood vessels and a consequent drop in the glomerular filtration rate [1, 27]. L-NAME (100 mg/kg) did indeed mimic the effect of MIBG/glucose on [^{51}Cr]-EDTA clearance at 5 h after injection, with partial recovery being observed after 24 h (Table 3). In contrast to MIBG, L-NAME increased renal perfusion from 11 ± 3% to 22 ± 5% ^{86}Rb/g tissue (Table 3). Similar results were obtained with a dose of 200 mg/kg L-NAME (18 ± 2% ^{86}Rb/g tissue), whereas 40 mg/kg L-NAME showed no effect (11 ± 4% ^{86}Rb/g tissue). Moreover, histological damage induced by L-NAME included prominent hypertrophy of the Bowman's capsule epithelium, which was more pronounced than that caused by MIBG, but vacuolization of tubular epithelial cells was only minor.

MIBG has structural similarities with L-arginine (Fig. 1) and may therefore, like L-NAME, act as a false substrate for NOS. To test this hypothesis we measured the production of [^{3}H]-L-citrulline after incubation of [^{3}H]-L-arginine, purified human NOS (I, II, III), and cofactors in the absence or presence of MIBG. MIBG (100 μ*M*) inhibited L-citrulline production at a substrate concentration of 50 μ*M* L-arginine for NOS-I, II, and III, and 1 m*M* MIBG further reduced L-citrulline production (Fig. 4). Similar results were obtained with 10 μ*M* L-arginine or with the crude enzyme in cell lysates from baculovirus-infected cells (data not shown).

Antagonism of MIBG-induced renal effects

We next tested whether the sympathomimetic effects of MIBG on kidney function could be antagonized by the α_1 receptor-blocker prazosin and the serotonin (5-HT_2) receptor-blocker cyproheptadine. Prazosin (4 mg/kg) and cyproheptadine (1 mg/kg) were capable of maintaining normal renal perfusion if given 1 h

Table 3 Renal perfusion (^{86}Rb distribution) and renal clearance of [^{51}Cr]-EDTA as determined at 3 or 24 h after treatment. [^{51}Cr]-EDTA (10 μCi/mouse) was injected i.p. at 30 min before blood sampling and killing of the animals

	Renal perfusion as % ^{86}Rb/g tissue (*n*) *t* = 3 h	[^{51}Cr]-EDTA clearance as % [^{51}Cr]-EDTA/ml plasma (*n*)	
		t = 3 h	*t* = 24 h
PBS	11 ± 3 (12)	1.9 ± 0.3 (7)	1.4 ± 0.4 (6)
MIBG 40 mg/kg + glucose 1.5 g/kg	6 ± 2** (12)	4.0 ± 1.6** (6)	4.3 ± 3.0* (6)
L-NAME 100 mg/kg	22 ± 5* (3)	5.1 ± 1.2** (8)	2.6 ± 0.4** (5)

*$P < 0.05$ versus control; **$P < 0.01$ versus control

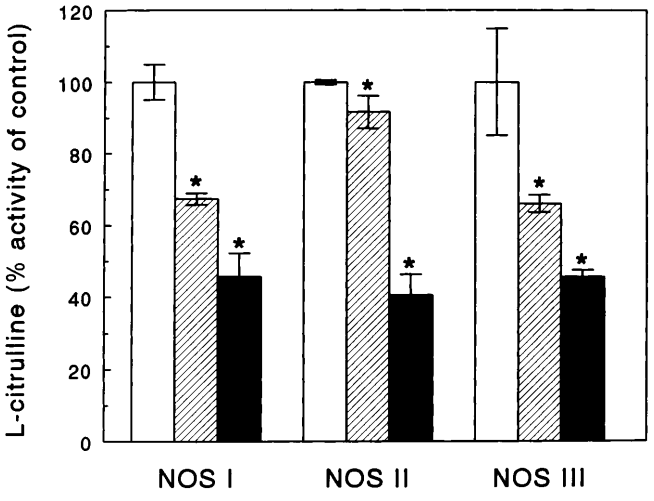

Fig. 4 Production of L-citrulline by purified human NOS I, II, and III in the presence of MIBG and 50 μ*M* L-arginine. (□ Controls set at 100%, ▨ 100 μ*M* MIBG, ■ 1 m*M* MIBG) Data represent mean values ± SD for 3 experiments; * $P < 0.05$ versus control

before or after MIBG/glucose treatment (Table 4). Prazosin and cyproheptadine alone slightly increased renal perfusion (Table 4), which was accompanied by increased liver perfusion, probably as a result of increased cardiac output. By contrast, effects on renal clearance were not prevented by either prazosin or cyproheptadine despite normalized renal perfusion (Table 4). The combination of either drug with MIBG/glucose treatment even further reduced renal clearance, whereas prazosin and cyproheptadine alone had no effect on the residual [^{51}Cr]-EDTA levels. Moreover, prazosin did not prevent the development of histological kidney damage.

We also investigated whether Ca^{2+}-channel antagonists could prevent the renal damage caused by MIBG. MIBG/glucose treatment increased the residual [^{51}Cr]-EDTA/ml plasma values from 1.7 ± 0.6% to 4.2 ± 1.0% ($P < 0.05$) and the Ca^{2+}-channel antagonist verapamil, given at 3 h before MIBG/glucose treatment, could neither prevent histological damage nor significantly ($P > 0.1$) improve [^{51}Cr]-EDTA clearance (residual [^{51}Cr]-EDTA plasma levels were 3.5 ± 1.5% and 3.8 ± 1.1% for combinations with 1 and 10 mg/kg verapamil, respectively). In a pilot experiment the Ca^{2+}-channel antagonist nifedipine (1 mg/kg), given at 3 h before MIBG/glucose treatment, resulted in a further increase in [^{51}Cr]-EDTA residual plasma activity to 5.0%.

The diuretics amiloride and bendroflumethiazide were given 3 h before MIBG/glucose and increased hematocrit levels by 15% and 11%, respectively, indicating effective diuresis at the doses used. Nevertheless, amiloride and bendroflumethiazide caused a further and significant increase ($P < 0.05$) in residual [^{51}Cr]-EDTA plasma levels when given at 3 h before MIBG/glucose treatment (10 mg/kg amiloride 7.4 ± 1.1% [^{51}Cr]-EDTA/ml plasma; 5 mg/kg bendroflumethiazide 5.9 ± 0.7% [^{51}Cr]-EDTA/ml plasma).

Effects of the MIBG analog BG

A comparison between MIBG and BG was made to assess the relative contribution of mitochondrial inhibition to renal damage. As in a previous study [7], MIBG/glucose caused an increase in plasma glucose levels (Fig. 5a). By contrast, BG treatment reduced plasma glucose levels, even when extra glucose was given simultaneously (Fig. 5b). Plasma lactate levels showed a strong increase in BG-treated mice but only a marginal rise in MIBG-treated mice (Fig. 5). Glucose alone (3 g/kg) did not affect plasma levels of either glucose or lactate (data not shown).

BG given alone or with extra glucose decreased renal perfusion to the same extent as did MIBG but had no effect on [^{51}Cr]-EDTA clearance (Table 5). Combination of BG with glucose slightly increased plasma [^{51}Cr]-EDTA levels, but only a sublethal dose of BG (60 mg/kg) equaled MIBG's effects on renal clearance (4.2 ± 1.7% [^{51}Cr]-EDTA/ml plasma). As judged from histological analysis, BG also caused some acute degeneration of cells of the corticomedullar tubuli and slight hypertrophy of Bowman's capsule. However, the overall level of histological damage induced by BG was much less pronounced than that caused by MIBG.

Direct cytotoxicity of MIBG to L1210 and renal cells

Since impaired renal function was clearly associated neither with vascular effects nor with mitochondrial inhibition, we assessed possibility of direct cytotoxicity of MIBG. To this end the single-cell survival of L1210 mouse lymphoma cells and the viability of primary

Table 4 Renal perfusion (^{86}Rb distribution) and [^{51}Cr]-EDTA clearance as determined at 3 h after MIBG/glucose treatement. Prazosin and cyproheptadine were given 2 h before the measurements. [^{86}Rb]-Cl and [^{51}Cr]-EDTA were injected at 1 and 30 min, respectively, before blood sampling and killing of the animals

	Renal perfusion as % ^{86}Rb/g tissue (*n*)	[^{51}Cr]-EDTA clearance as % [^{51}Cr]-EDTA/ml plasma (*n*)
PBS	11 ± 3 (12)	2.3 ± 0.7 (12)
MIBG 40 mg/kg + glucose 1.5 g/kg	7 ± 2*[a] (10)	4.6 ± 1.2*[a] (12)
Prazosin 4 mg/kg	15 ± 2*[a] (7)	2.1 ± 0.5 (7)
MIBG/glucose + prazosin (1 h after)	12 ± 2*[b] (5)	7.5 ± 0.3*[a] (7)
Cyproheptadine 1 mg/kg	14 ± 2*[a] (6)	2.2 ± 0.5 (4)
MIBG/glucose + cyproheptadine (1 h after)	12 ± 2*[b] (4)	5.8 ± 1.3*[a] (5)

*[a] $P < 0.01$ versus *PBS*-treated controls; *[b] $P < 0.01$ versus MIBG/glucose treatment alone

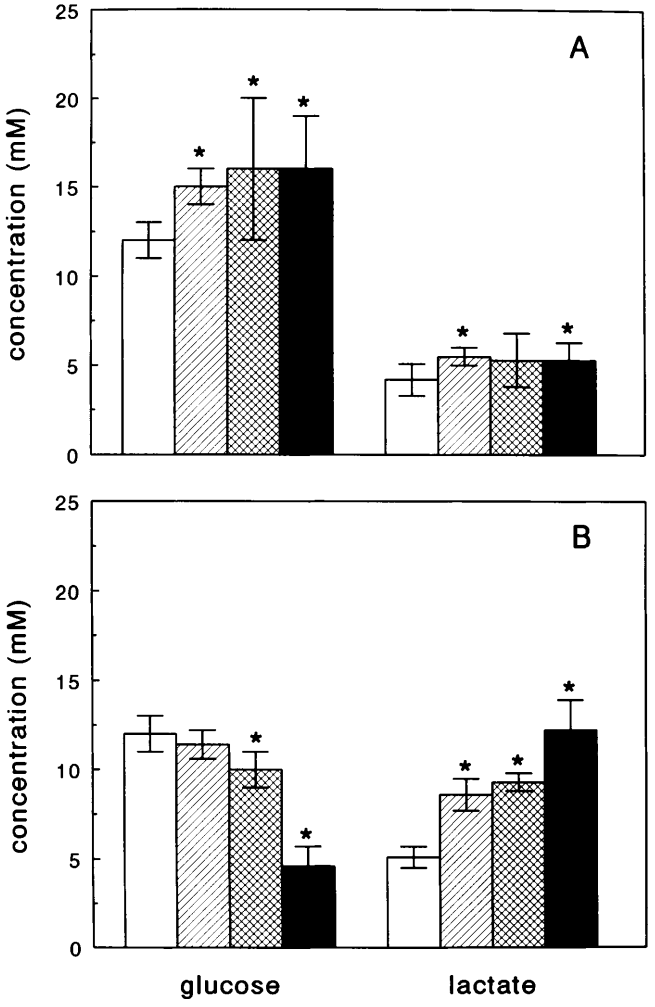

Fig. 5A,B Plasma glucose and lactate concentrations in animals treated with **A** MIBG (40 mg/kg) or **B** BG (43 mg/kg). (□ PBS-treated mice, ▨ MIBG or BG alone, ▩ MIBG or BG plus 1.5 g/kg glucose, ■ MIBG or BG plus 3.0 g/kg glucose) Data represent mean values ± SD for 6–8 mice; * $P < 0.05$ versus control

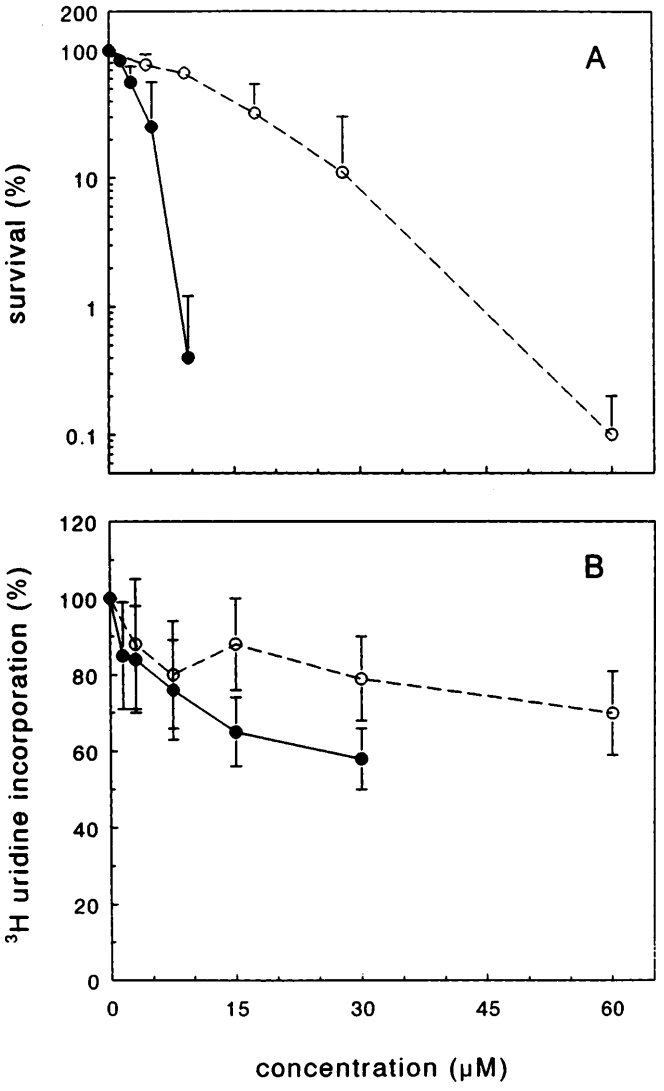

Fig. 6A,B Cytotoxicity of MIBG (●) and BG (○). **A** Single-cell survival of L1210 cells plated in media containing increasing concentrations of the drugs. **B** [^{3}H]-uridine incorporation in primary kidney cells of C3H/Km mice exposed for 12 or 24 h to MIBG or BG (pooled data). Data represent mean values ± SD for 3 individual experiments

mouse kidney cells were assayed as affected by MIBG or BG. The results depicted in Fig. 6a show that MIBG was much more cytotoxic than BG to L1210 cells, with IC_{10} values being 6.3 and 28.8 μ*M*, respectively. The metabolic activity of the primary kidney cells as measured by [^{3}H]-uridine incorporation was also more strongly affected by MIBG than by BG (Fig. 6b), which was not due to cell loss according to the unchanged protein content determined in control and treated cultures.

Discussion

MIBG has demonstrated promising results in the palliation of carcinoid syndromes [21] and is effective on a predosing schedule in increasing the relative tumor/tissue ratios resulting from radiolabeled MIBG treatment

Table 5 Renal perfusion (^{86}Rb distribution) and [^{51}Cr]-EDTA clearance as determined at 3 h after treatment. [^{86}Rb]-Cl and [^{51}Cr]-EDTA were injected at 1 and 30 min, respectively, before blood sampling and killing of the animals

	Renal perfusion as % ^{86}Rb/g tissue (*n*)	[^{51}Cr]-EDTA clearance as % [^{51}Cr]-EDTA/ml plasma (*n*)
PBS	12 ± 3 (15)	1.7 ± 0.5 (19)
MIBG 40 mg/kg	8 ± 2* (7)	2.5 ± 1.3 (8)
MIBG 40 mg/kg + glucose 1.5 g/kg	8 ± 3* (11)	4.9 ± 2.8* (10)
BG 43 mg/kg	9 ± 2* (14)	2.0 ± 0.4* (11)
BG 43 mg/kg + glucose 1.5 g/kg	9 ± 4 (6)	2.7 ± 0.8* (5)

*$P < 0.05$ versus control

of neuroendocrine tumors [13, 22]. For these and other applications, MIBG is being given to patients at doses of up to 135 mg/m^2 in ongoing dose-escalation studies (personal communication B. Taal, The Netherlands Cancer Institute), necessitating preclinical investigation of its possible side effects.

In the present report we describe changes in renal clearance, reduced renal perfusion, and histological kidney damage as being prominent but reversible side effects of high-dose MIBG. Co-administration of glucose only enhanced the MIBG-induced reduction in renal function, and the diuretic effect of increased glucose excretion was most probably the cause of the increased urinary volume measured after MIBG/glucose treatment (Table 1).

Since clearance of [^{51}Cr]-EDTA is dependent on glomerular filtration only, reduced renal perfusion as measured by ^{86}Rb distribution would seem a plausible cause of MIBG nephrotoxicity. However, full antagonism of perfusion effects by the catecholamine α_1 receptor-blocker prazosin and the 5-HT receptor-blocker cyproheptadine neither restored the glomerular filtration rate nor prevented histological damage. Apparently, reduction of perfusion, but not impaired renal clearance and tissue damage, is associated with sympathomimetic responses via MIBG-induced release of bioamines [7].

The persistent histological damage observed after antagonism of reduced renal perfusion was suggestive of local ischemia induced by possible hemodynamic effects of MIBG that are not detectable by ^{86}Rb distribution. L-NAME, which is known to reduce glomerular filtration via vasoconstriction of the afferent blood vessels [27], inhibited renal clearance to a similar extent as did MIBG, although the histological damage was more prominent in the glomeruli than in the distal tubuli. Rather than inhibiting renal perfusion, L-NAME increased it, confirming the inability of the ^{86}Rb assay to detect local vasoconstriction of afferent blood vessels. The increase in renal ^{86}Rb levels might reflect a systemic effect of L-NAME, namely, an increase in cardiac output [14].

Since the diamine group of MIBG has structural similarities with L-arginine, MIBG might mimic L-NAME as a false substrate for NOS. MIBG inhibited all three isoforms of human NOS, albeit at concentrations much higher than the maximal plasma levels of 37 μ*M* MIBG observed after a dose of 40 mg/kg, as estimated from biodistribution studies in this laboratory. However, because MIBG accumulates in metabolically active cells [17], the drug, like L-NAME, has at least the potential of reducing renal clearance via NOS inhibition [27].

Several studies have shown that the Ca^{2+}-channel blockers verapamil and nifedipine can prevent ischemic damage when given prior to clamping of blood vessels [12]. The failure of both drugs to prevent MIBG-induced kidney damage supports the conclusion that ischemic damage is not a major mechanism of the nephrotoxicity of MIBG. An alternative protective approach, namely, inhibition of the activity of the distal tubular cells by the diuretics amiloride and bendroflumethiazide, was also ineffective.

The analog BG given at twice the molar dose of MIBG did not decrease renal clearance and induced histological damage less pronounced than that caused by MIBG. The high plasma lactate levels observed after BG treatment confirmed its equipotent antimitochondrial effects in comparison with MIBG. Accordingly, mitochondrial inhibition is not primarily responsible for MIBG-induced renal impairment. The effects of BG on perfusion and reduced renal clearance at the sublethal dose of 60 mg/kg may be due to a combination of sympathomimetic effects and acidosis [19].

Cytotoxicity assays in primary cultures of both the tubular and the unrelated L1210 cells demonstrated that MIBG was intrinsically more cytotoxic than BG. The difference in cytotoxicity cannot be explained by shared antimitochondrial effects. The mechanism of cytotoxicity of MIBG is unknown but may be related to the previously described capacity of MIBG to inhibit mono(ADP-ribosylation) [8, 16], a process with an as yet unrevealed biological role. It could be argued that the in vivo exposures to MIBG do not match the time and dose conditions of the in vitro studies. Moreover, MIBG is not selectively toxic to kidney cells as determined in this investigation and a previous study [15], and drug levels in the kidney are not increased relative to those in other organs (unpublished results). However, in humans and mice, MIBG is primarily excreted by the kidney; within 24 h, about 60% of the injected dose ([27], M. Rutgers, unpublished results), i.e., 1.2 mg/mouse weighing 30 g, will be excreted in a urinary volume of 1.8 ml (Table 1). Accordingly, the average MIBG concentration in urine will be approximately 1.2 m*M*, exposing the distal tubuli to concentrations about 100-fold the concentrations that cause detectable effects in vitro. Therefore, a direct cytotoxic effect of MIBG on tubular cells could plausibly explain the observed functional and structural kidney damage and the failure of the protective interventions tested thus far.

In summary, our results confirm previous findings that MIBG causes stress-related symptoms that are associated with the release of bioamines [7] and affect renal perfusion. Appropriate bioamine-receptor blockade by prazosin or cyproheptadine antagonized reduced renal perfusion and could therefore be applied for the management of corresponding MIBG side effects observed in the clinic ([21]; personal communication, B. Taal, The Netherlands Cancer Institute). The stress-related reduction in renal perfusion was fully dissociable from MIBG's effects on renal clearance and on histological damage to the distal tubuli. The latter effects could not be explained by inhibition of mitochondrial respiration but were most probably due to direct cytotoxicity of MIBG, with a possible contribution of local vasoconstriction via NOS inhibition.

Although renal effects were reversible in the present investigation and have not yet been encountered in

dose-escalation studies in patients, on a predosing schedule, MIBG might reduce the glomerular filtration rate, which could decrease the clearance of therapeutic [^{131}I]-MIBG [25]. Likewise, the pharmacokinetics of concomitant anticancer drugs might be affected by MIBG so as to increase their toxicity. Therefore, effects on renal function observed after escalation and frequent application of MIBG should be taken into account.

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