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Meta-iodobenzylguanidine (MIBG) Inhibits Malate and Succinate Driven Mitochondrial ATP Synthesis in the Human Neuroblastoma Cell Line SK-N-BE(2c)

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In this paper, we report on our studies of the effects of MIBG, a structural analogue of norepinephrine, on SK-N-BE(2c) cells. In micromolar concentrations, MIBG caused almost complete inhibition of the proliferation of SK-N-BE(2c) cells. In intact SK-N-BE(2c) cells, addition of MIBG led to a decrease of the ATP to ADP ratio. A progressive increase of the lactate to pyruvate ratio (due to increased lactate production) was observed after incubation of the cells with glucose and increasing concentrations of MIBG. In cells treated with digitonin, MIBG inhibited malate driven ATP synthesis. Comparable inhibition of ATP synthesis with succinate as a substrate required higher concentrations of MIBG. These results indicate that, apart from inhibition of complex I, MIBG was capable of inhibiting at least one other complex of the respiratory chain. Although maximal inhibition of ATP synthesis was observed at a concentration of 10 μM, optimal inhibition of cell proliferation occurred at a MIBG concentration > 25 μM. This suggests that MIBG also influences other cellular processes apart from mitochondrial ATP synthesis, resulting in additional inhibition of cell proliferation.

Key words: meta-iodobenzylguanidine, respiratory chain, SK-N-BE(2c), ATP synthesis, neuroblastoma
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INTRODUCTION

META-IODOBENZYLGUANIDINE (MIBG) is a structural and functional analogue of the neurotransmitter norepinephrine. Owing to these properties, MIBG is capable of competing with norepinephrine for uptake in tissues of neuroendocrine origin [1]. In its radio-iodinated form ([^{131}I]MIBG), MIBG is used as a radiopharmaceutical for the imaging and targeted radiotherapy of neuroendocrine tumours, such as pheochromocytoma and neuroblastoma [2, 3]. Unlabelled MIBG is known to have antiproliferating effects against animal tumours and a large number of cell lines of various origin [4]. Recently, several patients with carcinoid tumours (neuroendocrine tumours capable of MIBG uptake) participated in a phase II trial, in which high doses of unlabelled MIBG were administered for potential palliative therapy (Dr B.G. Taal, Antonie v. Leeuwenhoek Hospital).

It is thought that at least part of the antiproliferating effect of MIBG is due to inhibition of complex I of the mitochondrial respiratory chain [5, 6]. In rat liver mitochondria, state-3 oxidation of malate was inhibited in the presence of MIBG, whereas state-3 oxidation of succinate was unaffected. In the human neuroblastoma cell line SK-N-SH, and the murine cell lines, S 49 (lymphosarcoma) and N_1E115 (neuroblastoma), the presence of MIBG led to an enhanced glycolytic flux, decreased ATP levels and decreased oxygen consumption, suggesting inhibition of mitochondrial respiration [6].

So far, no direct evidence has been presented that demonstrates inhibition by MIBG of mitochondrial ATP synthesis in human neuroblastoma cells. Therefore, we studied the effects of MIBG on cell proliferation and several parameters related to mitochondrial respiration in the human neuroblastoma cell line SK-N-BE(2c) [7, 8], a cell line capable of MIBG uptake.

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MATERIALS AND METHODS

Cell culture

The human neuroblastoma cell line SK-N-BE(2c) [7, 8] was cultured at 37°C in a humidified atmosphere of 95% air with 5% CO_2. DMEM containing pyruvate (Gibco, Paisley, Scotland), supplemented with 10% fetal calf serum (Sebak GmbH, Aidenbach, Germany), 10 mM L-glutamine (Gibco, Paisley, Scotland), 0.1 mg/ml penicillin and 100 IU streptomycin/ml (Gibco, Paisley, Scotland) was used as culture medium.

The cells were cultured in the presence of adequate concentrations of MIBG (Cis Bio international, Gif-sur-Yvette, France) for 12 h, in order to study the effects of MIBG on energy

metabolism. In the studies on the effect on cell proliferation, MIBG was continuously present in the culture medium.

ATP to ADP ratio in SK-N-BE(2c) cells

SK-N-BE(2c) cells were cultured in 10 cm^2 wells. Before nucleotide extraction, the cells were washed with 2 ml of ice-cold phosphate buffered saline. Nucleotides were extracted at 4°C in 200 μl of 0.4 M perchloric acid (PCA). After removal of the precipitated protein, the extracts were neutralised with 2 M potassium hydroxide plus 0.6 M 3-[N-morpholino]propanesulphonic acid (MOPS). ATP and ADP analysis by HPLC was conducted according to the method of de Korte and associates [9]. Standard mixtures containing known amounts of ATP and ADP were used for calibration.

Lactate to pyruvate ratio in SK-N-BE(2c) cells

Measurement of lactate and pyruvate production from glucose in SK-N-BE(2c) cells was essentially performed according to the method of Wijburg and associates [10], with one minor modification: a 1.5 h incubation with glucose, instead of 4 h.

ATP synthesis in SK-N-BE(2c) cells

Measurement of ATP synthesis in SK-N-BE(2c) cells with malate or succinate as a substrate was essentially performed according to the method of Wanders and colleagues [11]; cells permeabilised with digitonin (30 μg/ml) were incubated for 30 min at 25°C in a medium containing K_2HPO_4/KH_2PO_4 buffer (pH 7.4), KCl, EDTA, ADP, Tris–HCl buffer (pH 7.4) and either malate (in the presence of glutamate) or succinate (in the presence of rotenone). Under these conditions, all cytosolic ATPases remain inactive, as Mg^{2+} (essential for all ATPase activities) or Na^+ (essential for the Na^+/K^+ ATPases) are not available. After 30 min, the reactions were terminated with PCA and ATP was determined fluorometrically.

RESULTS

Effects of MIBG on cell proliferation of SK-N-BE(2c) cells

MIBG is known to affect cell proliferation of a large number of cell lines of both neuroblastoma and other origin. In order to study the effects of MIBG on the proliferation of SK-N-BE(2c) cells, the cells were cultured in the continuous presence of various concentrations of MIBG. After 4 days, the cell cultures were harvested and the protein content measured. The number of cell divisions, a measure for proliferation, was calculated by comparing the protein content of these cultures with the protein content at the beginning of the experiment (day 0). Figure 1 shows that the presence of MIBG had a negative effect on cell proliferation, maximal effects were reached at a MIBG concentration of approximately 25 μM.

Effect of MIBG on the ATP to ADP ratio in SK-N-BE(2c) cells

In order to study the effect of MIBG on the energy status of the SK-N-BE(2c) cells, we measured the ATP to ADP ratio in these cells after culturing for 12 h in the presence of MIBG. As is shown in Figure 2, addition of MIBG led to a decrease of the ATP to ADP ratio in the SK-N-BE(2c) cells. The ATP to ADP ratio decreased from approximately 14 under standard conditions (i.e. no MIBG added) to a minimum value of approximately 5 in the presence of MIBG. The maximal effect on the ATP to ADP ratio was achieved at a MIBG concentration of 1 μM.

Effects of MIBG on lactate to pyruvate ratio

Measurement of the rates of production of pyruvate and lactate from glucose in intact cells can yield valuable information

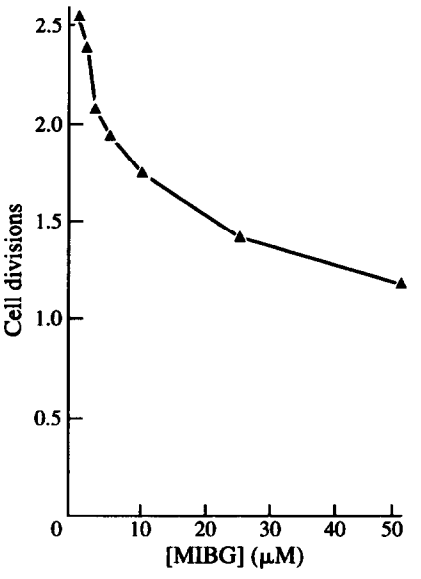

Figure 1. Effect of MIBG on proliferation of SK-N-BE(2c) cells. SK-N-BE(2c) cells were cultured in DMEM culture medium in the presence of various concentrations of MIBG. After 4 days, cells were harvested and the number of cell divisions determined by measuring the protein content of the cultures. Results are given as the mean of 2 independent experiments.

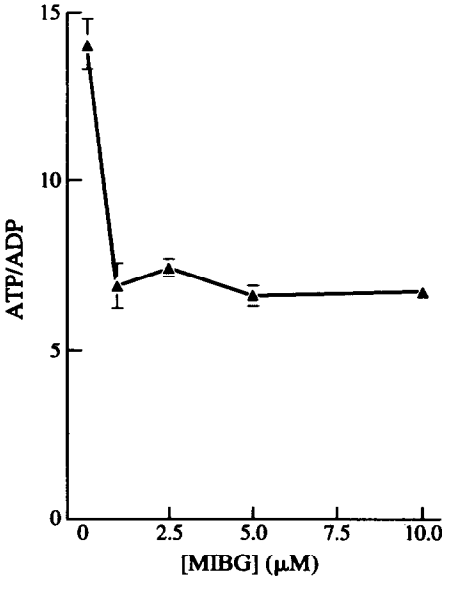

Figure 2. Effect of MIBG on the ATP to ADP ratio of SK-N-BE(2c) cells. SK-N-BE(2c) cells were cultured in the presence of various concentrations of MIBG. After 12 h, nucleotides were extracted with 0.4 M PCA, and ATP and ADP concentrations determined by HPLC. Results are given as the mean of 3–6 independent experiments ±S.D..

on the functional integrity of the respiratory chain. In order to study the effect of MIBG on the respiratory chain of SK-N-BE(2c) cells, we measured the lactate and pyruvate production from 10 mM glucose in the presence of various concentrations of MIBG. In Figure 3 it can be seen that increasing concentrations of MIBG led to a progressive increase of the lactate to pyruvate ratio, resulting in a maximal ratio at 5 μM MIBG. The increase of the lactate to pyruvate ratio was predominantly due to an increase of lactate production. The maximal lactate to pyruvate ratios observed were in the same order of magnitude as

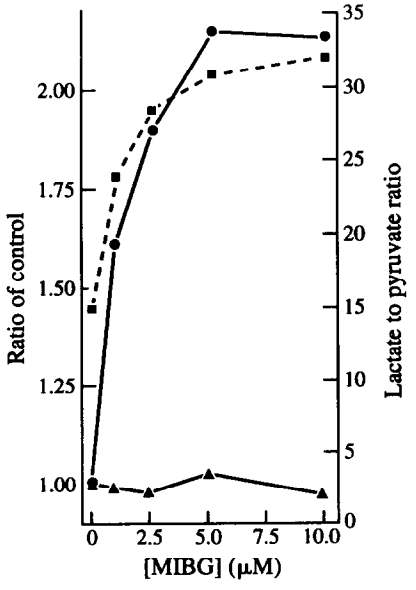

Figure 3. Lactate and pyruvate production in SK-N-BE(2c) cells after incubation with 10 mM glucose and various concentrations of MIBG. SK-N-BE(2c) cells were incubated in the presence of 10 mM glucose and various concentrations of MIBG. After 1.5 h, the reactions were terminated and concentrations of lactate and pyruvate determined as described in Materials and Methods. Results are given as the mean of 2 independent experiments and represent, on the left *y*-axis the lactate or pyruvate production as a ratio of control values, i.e., no MIBG added (452 nmol lactate/mg/h and 30 nmol pyruvate/mg/h) and on the right *y*-axis the lactate to pyruvate ratio. -●-, lactate production; -▲-, pyruvate production; -■-, lactate to pyruvate production.

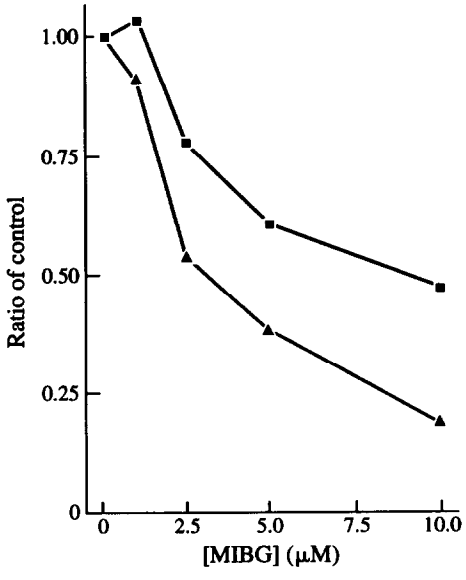

Figure 4. ATP synthesis in SK-N-BE(2c) cells with either malate or succinate as substrates in the presence of various concentrations of MIBG. Digitonin permeabilised SK-N-BE(2c) cells were incubated in the presence of 10 mM malate or succinate and various concentrations of MIBG. After 30 min, the reactions were terminated and ATP concentrations determined as described in Materials and Methods. Results are given as the mean of 2 independent experiments, and represent the ratio of control values, i.e., no MIBG added (771 nmol ATP/mg/h with malate as substrate and 179 nmol ATP/mg/h with succinate as substrate). -▲-, malate driven ATP synthesis; -■-, succinate driven ATP synthesis.

those observed when rotenone or antimycin were added in concentrations (20 μg/ml) that completely inhibited the activity of complex I or III, respectively (data not shown).

Effects of MIBG on ATP synthesis in SK-N-BE(2c) cells

In order to study the effects of MIBG on oxidative phosphorylation, we measured ATP synthesis as a result of state-3 oxidation of malate and succinate, in the presence of various concentrations of MIBG. ATP synthesis of digitonin treated SK-N-BE(2c) cells was inhibited by MIBG in a dose-dependent manner when either malate (in the presence of glutamate) or succinate (in the presence of rotenone) was used as a substrate for oxidative phosphorylation. This suggests that apart from complex I, at least one other complex is inhibited by MIBG. The degree of inhibition observed, when succinate was used as a substrate for ATP synthesis, was significantly lower as compared with that observed when malate was used as a substrate.

DISCUSSION

This paper presents our studies on the effects of MIBG on cell proliferation and mitochondrial respiration in SK-N-BE(2c) cells. The SK-N-BE(2c) cell line was chosen because SK-N-BE(2c) cells are capable of active MIBG uptake and unlike the widely used cell line SK-N-SH, are *MYCN* amplified and grow relatively rapidly [7, 8].

In DMEM culture medium, increasing concentrations of MIBG led to a dose-dependent inhibition of proliferation. At a concentration of approximately 25 μM, maximal inhibition of proliferation of SK-N-BE(2c) cells was reached. Gaze and colleagues reported that MIBG at a concentration of 2 mM had no effect on cell proliferation of SK-N-BE(2c) cells [12]. However, in their experiments, cells were cultured as multicellular spheroids (obtained by continuous stirring of a trypsin dispersed monolayer culture). Spheroids thus obtained are probably anoxic and have to rely on glycolytic ATP production. This is most likely the explanation why the cells did not show a decrease in proliferation rate after addition of MIBG in concentrations that caused complete inhibition of mitochondrial ATP synthesis in our experiments.

Measurement of the rates of production of pyruvate and lactate from glucose, a method which is used to identify defects in the oxidative phosphorylation system [13], showed that increasing concentrations of MIBG led to a progressive increase of the lactate to pyruvate ratio, caused by an increase in lactate production. Increase of the lactate to pyruvate ratio is probably a result of the inhibition of mitochondrial respiration. Loesberg and associates [6] reported an enhanced glycolytic flux in various cell types after addition of MIBG to the culture medium. They concluded that this was due to glycolytic compensation for inhibition of mitochondrial respiration. An increased glycolytic flux is accompanied by increased lactate production (and a reduced pH). As a number of anticancer drugs have a higher cytotoxicity at acidic pH, various strategies to improve the therapeutic index of these drugs are based on this phenomenon. *In vivo*, specific stimulation of glycolysis in tumours by MIBG leads to a reduced pH in these tumours. A further pH reduction is achieved by moderately increasing the glucose concentration in the plasma [14, 15]. Therefore, the combination of MIBG and these anticancer drugs might improve the therapeutic index of these drugs.

When SK-N-BE(2c) cells were grown for 12 h in the presence of MIBG, mitochondrial ATP synthesis with malate as well as with succinate as substrate was inhibited. Succinate driven ATP

synthesis, however, was affected to a lesser extent, indicating that MIBG inhibits at least one additional complex of the respiratory chain in addition to complex I. Measurement of the individual respiratory chain complexes in the lymphoblastic leukaemia cell line Molt-4, revealed that apart from complex I, complex III is the other complex inhibited in the presence of MIBG [16]. The effect of MIBG on mitochondrial respiration is likely to be a general one rather than an effect specific for a certain cell type. Therefore, it is likely that the inhibition of succinate driven ATP synthesis in SK-N-BE(2c) cells is due to inhibition of the activity of complex III.

Loesberg and associates [7] did not find a clear effect of low concentrations of MIBG on the activity of oxidative phosphorylation with succinate as a substrate which might be explained by the short (2 min) preincubation period with MIBG used in their experiments. MIBG can enter the cell either by passive diffusion (a relatively slow process), or by active uptake (uptake 1 mechanism). Mitochondria do not possess the active uptake system, so uptake of MIBG in the mitochondria will therefore take place by passive diffusion. Import studies in the murine lymphoblastic leukaemia cell line L1210 (incapable of active uptake), revealed that saturation with MIBG through passive diffusion takes approximately 3 h [17]. Alternatively, cell lines that do possess the active uptake system, show maximal intracellular MIBG concentrations after a 1–2 h incubation period with MIBG [18, 19]. However, at relatively high MIBG concentrations ($> 10^{-6}$ M), passive diffusion dominates over active uptake as the uptake proteins become saturated [20]. Therefore, we used a preincubation period with MIBG of at least 4 h, in the experiments in which the effects of MIBG on energy metabolism were studied.

Malate driven ATP synthesis was inhibited to a greater extent by MIBG as compared with the inhibition of ATP synthesis with succinate as a substrate. This can easily be explained, since inhibition of the activity of both complexes I and III by MIBG contribute to the total inhibition of ATP synthesis when malate is used as a substrate for ATP synthesis, whereas inhibition of ATP synthesis with succinate as a substrate is only due to inhibition of complex III.

The concentrations of MIBG at which inhibition of mitochondrial ATP synthesis occurs are much higher than the recorded peak plasma levels (0.1 μM), obtained when neuroblastoma patients are treated with [^{131} I]MIBG (Prof. Dr P.A. Voûte, Academic Medical Centre). Since MIBG accumulates 30-fold in neuroblastoma cells [21], the theoretical MIBG concentration within the tumour will be approximately 3 μM. It is, therefore, unlikely that the cytotoxic effects described in this paper contribute substantially to the effects observed on neuroblastoma *in vivo*. Recently, however, pilot studies have been performed in which high doses (approximately four times a [^{131} I]MIBG dose) of unlabelled MIBG were given to carcinoid patients (Dr B.G. Taal, Antonie v. Leeuwenhoek Hospital). Under these conditions, MIBG concentrations within the tumour are theoretically similar to concentrations leading to serious inhibition of mitochondrial ATP synthesis described in this paper, and may have antitumour effects as described earlier by Smets and associates [4].

An intriguing phenomenon is the observation that the activity of the mitochondrial respiratory chain is almost completely inhibited in the presence of 10 μM MIBG, but only minor effects on the proliferation of the SK-N-BE(2c) cells were observed at this MIBG concentration. However, the cells were cultured in a medium in which inhibition of mitochondrial function alone will hardly affect proliferation [22]. Therefore, optimal arrest of proliferation at MIBG concentrations of 25 μM suggests that MIBG influences other processes in addition to mitochondrial oxidative phosphorylation, resulting in a decreased proliferation of the SK-N-BE(2c) cells.

Mono-ADP ribosylation, a covalent modification reaction of proteins, is another cellular process known to be inhibited by MIBG [17, 23]. The exact role of mono-ADP ribosylation is not yet clear, although it is likely that it plays a role in cellular signal transduction or metabolic regulation [24]. Further research needs to be carried out to reveal whether inhibition of mono-ADP ribosylation or other processes are causing the non-mitochondrial effect of MIBG on cell proliferation.

1. Wieland DM, Wu JI, Brown LW, Magner TJ, Swanson DP, Beierwaltes WH. Radiolabeled adrenergic neuroblocking agents: adrenomedullary imaging with [^{131}I] iodobenzylguanidine. *J Nucl Med* 1980, **21**, 349–353.
2. Hoefnagel CA, Voûte PA, de Kraker J, Marcuse HR. Total-body scintigraphy with ^{131}I-meta-iodobenzylguanidine for detection of neuroblastoma. *Diagn Imag Clin Med* 1985, **54**, 21–27.
3. Hoefnagel CA, Voûte PA, de Kraker J, Marcuse HR. Radionuclide diagnosis and therapy of neural crest tumors using iodine-131 meta iodobenzylguanidine. *J Nucl Med* 1987, **28**, 308–314.
4. Smets LA, Bout B, Wisse J. Cytotoxic and anti-tumor effects of the norepinephrine analogue meta-iodo-benzylguanidine (MIBG). *Cancer Chemother Pharmacol* 1988, **21**, 9–13.
5. Loesberg C, van Rooy H, Romyn JC, Smets LA. Mitochondrial effects of the guanidino group containing cytostatic drugs m-iodobenzylguanidine and methylglyoxal bis (guanylhydrazone). *Biochem Pharmacol* 1991, **42**, 793–798.
6. Loesberg C, van Rooy H, Nooyen WJ, Meijer AJ, Smets LA. Impaired mitochondrial respiration and stimulated glycolysis by m-iodobenzylguanidine (MIBG). *Int J Cancer* 1990, **46**, 276–281.
7. Reynolds CP, Tomayko MM, Donner L, *et al.* Biological classification of cell lines derived from human extra-cranial neural tumors. In *Advances in Neuroblastoma Research* 2. New York, Alan R. Liss, 1988, 291–306.
8. Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS. Multiple neuron-transmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Res* 1978, **38**, 3751–3757.
9. De Korte D, Havekort WA, Roos D, Van Gennip AH. Anion exchange high performance liquid chromatogrophy method for quantitation of nucleotides in human blood cells. *Clin Chem Acta* 1985, **148**, 185–196.
10. Wijburg FA, Feller N, de Groot CJ, Wanders RJA. Menadione partially restores NADH-oxidation and ATP-synthesis in complex I deficient fibroblasts. *Biochem Int* 1990, **22**, 303–309.
11. Wanders RJA, Ruiter JPN, Wijburg FA. Studies on mitochondrial oxidative phosphorylation in permeabilized human skin fibroblasts: application to mitochondrial encephalomyopathies. *Biochim biophys Acta* 1993, **1181**, 219–222.
12. Gaze MN, Mairs RJ, Boyack SM, Wheldon TE, Barrett A. ^{131}I-meta Iodobenzylguanidine therapy in neuroblastoma spheroids of different sizes. *Br J Cancer* 1992, **66**, 1048–1052.
13. Robinson BH, Glerum DM, Chow W, Petrova-Benedict R, Lightowlers R, Capaldi R. The use of skin fibroblast cultures in the detection of respiratory chain defects in patients with lacticacidemia. *Pediat Res* 1990, **28**, 549–555.
14. Jähde E, Volk T, Atema A, Smets LA, Glüsenkamp KH, Rajewsky MF. pH in human tumor xenografts and transplanted rat tumors: effects of insulin,inorganic phosphate and *m*-iodobenzylguanidine. *Cancer Res* 1992, **52**, 6209–6215.
15. Kuin A, Smets LA, Volk T, *et al.* Reduction of intratumoral pH by the mitochondrial inhibitor *m*-iodobenzylguanidine (MIBG) and moderate hyperglycemia. *Cancer Res* 1994, **54**, 3785–3792.
16. Cornelissen J, Wanders RJA, Van Gennip AH, Van den Bogert C, Voûte PA, Van Kuilenburg ABP. Meta-Iodobenzylguanidine inhibits complex I and III of the respiratory chain in the human cell line Molt-4. *Biochem Pharmacol* 1995 (in press).
17. Smets LA, Loesberg C, Janssen M, Van Rooij H. Intracellular inhibition of mono (ADP-ribosylation) by *meta*-iodobenzylguanid-

ine: specificity, intracellular concentration and effect on glucocorticoid-mediated cell lysis. *Biochim biophys Acta* 1990, **1054**, 49–55.
18. Smets LA, Loesberg C, Janssen M, Ebtisam A, Metwalk A, Huiskamp R. Active uptake and extravesicular storage of meta-Iodobenzylguanidine in human neuroblastoma SK-N-SH cells. *Cancer Res* 1989, **49**, 2941–2944.
19. Iavarone A, Lasorella A, Servidei T, Riccordi R, Mastrangelo R. Uptake and storage of meta-Iodobenzylguanidine are frequent neuronal functions of neuroblastoma cell lines. *Cancer Res* 1993, **53**, 304–309.
20. Lashford LS, Hancock JP, Kemshead JT. Meta-Iodobenzylguanidine (mIBG) uptake and storage in the human neuroblastoma cell line SK-N-BE(2c). *Int J Cancer* 1991, **47**, 105–109.
21. Buck J, Bruchelt G, Girgert R, Treumer J, Niethammer D. Specific uptake of m[^{125}I]iodobenzylguanidine in the human neuroblastoma cell line SK-N-SH. *Cancer Res* 1985, **45**, 6366–6370.
22. Van den Bogert C, Spelbrink JN, Dekker HL. Relationship between culture conditions and the dependency on mitochondrial function of mammalian cell proliferation. *J Cell Physiol* 1992, **152**, 632–638.
23. Loesberg C, Van Rooij H, Smets LA. Meta-iodobenzylguanidine (MIBG), a novel high-affinity substrate for cholera toxin that interferes with cellular mono (ADP-ribosylation). *Biochim biophys Acta* 1990, **1037**, 92–99.
24. Bertazonni U, Scovassi AI, Shall S. Fourth European Meeting on ADP-ribosylation of proteins, Pavia (Italy). *Mutat Res* 1989, **219**, 303–307.

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Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis of Monoamine Transporters in Neuroblastoma Cell Lines: Correlations to Meta-iodobenzylguanidine (MIBG) Uptake and Tyrosine Hydroxylase Gene Expression

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Radiolabelled meta-iodobenzylguanidine (MIBG) has been widely used in scintigraphy and targeted radiotherapy in patients with neuroblastoma. Recently, it has been demonstrated that MIBG is incorporated into neuroblastoma cells by the noradrenaline transporter. *In vitro* experiments on SK-N-SH human neuroblastoma cells performed in the present study showed that uptake of MIBG is inhibited by noradrenaline, more so by dopamine and to a lesser extent, by serotonin, indicating that the respective transporters may also contribute to MIBG uptake. However, neither dopamine nor serotonin transporter gene expression was detected. Noradrenaline transporter gene expression was found in 4 of 6 investigated cell lines, which correlated with specific MIBG uptake. Furthermore, an inverse correlation of noradrenaline transporter and tyrosine hydroxylase gene expression, the key regulatory enzyme of catecholamine synthesis, was observed. These data show that MIBG is specifically incorporated only in neuroblastoma cells in which there is noradrenaline transporter gene expression. Furthermore, the catecholamine status in neuroblastoma cells is regulated by a coordinate expression of the key elements of catecholamine synthesis and reuptake systems.

Key words: neuroblastoma, MIBG uptake, noradrenaline transporter, tyrosine hydroxylase, gene expression, catecholamines

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