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EFFECTS OF METHYLMERCURIC CHLORIDE ON HIGH AFFINITY UPTAKE OF CERTAIN PUTATIVE NEUROTRANSMITTERS IN NEUROBLASTOMA AND GLIOMA CELL CULTURES

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SUMMARY

The effect of methylmercuric chloride (CH_3HgCl) on high affinity uptake of certain putative neurotransmitters on mouse neuroblastoma (NBE^-) and rat glioma (C-6) cells in culture 3 days after treatment was studied. The energy-dependent accumulation of radioactive choline was decreased in NB cells after treatment with CH_3HgCl (1 μM), whereas the accumulation of radioactive glycine or glutamate was increased. The uptake of radioactive glycine or glutamate in glioma cells was decreased after treatment with CH_3HgCl (0.3 μM). High affinity uptake of choline was not demonstrable in glioma cells. Low concentrations of CH_3HgCl affect the accumulation of glycine and glutamate in glioma and NB cells in an opposite manner.

INTRODUCTION

CH_3HgCl causes neurological disorders, referred to as Minamata Disease [1—3]. The cellular and molecular mechanisms of CH_3HgCl-induced damage to nervous tissue are unknown. 10 μM CH_3HgCl inhibits the high affinity uptake of certain putative neurotransmitters (dopamine, glycine, choline, glutamate and GABA) in adult mouse brain homogenates [4]. In rats, long-term administration of methylmercury decreases the levels of cortical acetylcholine and brainstem 5-hydroxytryptamine and norepinephrine [5]. In developing rat brain the concentrations of serotonin, norepinephrine, dopamine and 5-hydroxyindoleacetic acid were below control level within 24 h of exposure to CH_3HgCl [6]. A significant disturbance on neuromuscular transmission after administration of inorganic mercury has been observed [7—8]. These studies do not provide

Abbreviations: GABA, γ-aminobutyric acid; NB, neuroblastoma; PBS, Phosphate-buffered saline.

information on the response of neurons and glial cells separately. In the present study monolayer cultures of glioma and NB cells have been used. Although these cells are of tumor origin, and represent proliferative systems, they exhibit many features of mature glial cells and neurons, respectively. Previous studies have shown that: (a) glioma cells are more sensitive to CH_3HgCl than NB cells for the criteria of cell death and inhibition of cell division [9]; (b) the intracellular level of cyclic AMP increases after treatment of glioma (0.3 μM) and NB cells (1 μM) with CH_3HgCl [10]; (c) long-term treatment of glioma and NB cells with low concentrations of CH_3HgCl (0.1 μM) reduces the sensitivity of adenylate cyclase to prostaglandin E_1 in glioma cells, but not in NB cells [11], and produces marked alterations in gene expression in glioma cells and NB cells [12—13]. We now report that the treatment of glioma and NB cells with CH_3HgCl affects the uptake of certain putative neurotransmitters in a different way.

MATERIALS AND METHODS

Neuroblastoma clone (NBE^-) containing choline acetyltransferase but no tyrosine hydroxylase [14] was used. Rat glioma cells (C-6) [15] of passages 28—38 [16] were employed. Neuroblastoma cells were grown in F12 medium containing 10% agammaglobulin newborn calf serum, and glioma cells were grown in F12 containing 10% fetal calf serum. Both media contained penicillin (100 U./ml) and streptomycin (100 μg/ml). Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells (10^5) were plated in Lux tissue culture dishes (60 mm) and CH_3HgCl was added 24 h later. The drug and medium were changed 2 days after, and the uptake study was performed 3 days after treatment. Fresh growth medium (3 ml) without CH_3HgCl was added 30 min prior to the addition of radioactive putative neurotransmitters. In view of the greater sensitivity of glial cells to CH_3HgCl [9], they were cultured at a concentration of $0.3 \cdot 10^{-6}$ M while NB cells were grown in 10^{-6}M. 10 μl of radioactive compounds containing 0.5 μCi were then added to the dishes. Final μM concentrations of each compound used were L-[2,3-^{3}H]glutamic acid (17.5 Ci/mmol), $0.97 \cdot 10^{-8}$ M; [2-^{3}H]glycine (15.0 Ci/mmol) $1.1 \cdot 10^{-8}$ M; [methyl-^{3}H]choline (69.5 Ci/mmol) $0.25 \cdot 10^{-8}$ M; [2,3$^-$^{3}H]GABA (54 Ci/mmol) $0.31 \cdot 10^{-8}$ M; and [U$^-$^{3}H]dopamine (5.0 Ci/mmol) $3.4 \cdot 10^{-8}$ M.

The culture dishes were incubated at 37°C for 10 min while identically prepared dishes were maintained at 0°C for 10 min. Active transport was taken to be the difference in accumulation by 37°C incubated dishes and their cold-maintained counterparts. A short incubation time was chosen to minimize the possibility of further metabolism of transported compounds [17]. The dishes were rinsed 3 times with 5 ml cold PBS, care being taken not to dislodge the layer of cells adhering to the bottom of the plate. 1 ml 0.5% trypsin in water was then layered over to take the cells up into suspension which was then incubated (23°C, 30 min) and collected, and the plates were rinsed with 0.5 ml water. The combined trypsin and water washings were added to 15 ml 3a70

(Research Products Inc., Elk Grove, IL), a water compatible scintillation counting solution.

Radioactive assay was performed with a Beckman Liquid Scintillation spectrometer at an efficiency of 37—39%. Protein was determined by the method of Lowry et al. [18].

RESULTS AND DISCUSSION

Previous studies have demonstrated the presence of high affinity uptake of choline [19] and norepinephrine [20] in NB clones, but the high affinity uptake of dopamine has not been observed [21]. Our results demonstrate the presence of high affinity uptake of choline in a cholinergic NB clone (NBE^-), but not in glioma cells. The presence of energy-dependent uptake of glycine and glutamate in both NB and glioma cells in culture is shown.

Treatment of NB cells with 1 μM CH_3HgCl reduces the accumulation of choline in NB cells. The concentration of CH_3HgCl required to inhibit choline uptake in NB cells was 1/10 of that required for its inhibition in adult mouse brain homogenates [4]. The reduced uptake of choline may in part account for a decreased level of acetylcholine in the cortical area of rat brain [5]. The energy-dependent uptake of dopamine and GABA was not shown in NB cells, but was demonstrable in adult mouse brain homogenates [4], due in part perhaps to the fact that this clone of NB is of cholinergic type with relatively undifferentiated cells.

Treatment of NB (Table I) and of glioma cells (Table II) with low concentrations of CH_3HgCl produced a contrasting effect on the accumulation of glycine and glutamate. The energy-dependent accumulation of radioactive

TABLE I

EFFECT OF METHYLMERCURIC CHLORIDE (CH_3HgCl) ON THE ACTIVE ACCUMULATION OF PUTATIVE NEUROTRANSMITTERS IN MOUSE NEUROBLASTOMA CELLS IN (NBE^-) IN CULTURE

Cells (10^5) were plated in Lux culture dishes (60 mm), and CH_3HgCl was added 24 h later. Drug and medium were changed 2 days after treatment and the uptake study was performed 3 days after treatment. Experiments were repeated three times involving three dishes per putative neurotransmitter each time. Data are representative of one such experiment

Treatment	Neurotransmitters	pmoles accumulated/ min/100 mg protein
Control	Choline	8.9 ± 1.2
CH_3HgCl (1 μM)	Choline	5.1 ± 1.0
Control	Glutamate	1.2 ± 0.3
CH_3HgCl (1 μM)	Glutamate	4.1 ± 0.4
Control	Glycine	1.9 ± 0.4
CH_3HgCl (1 μM)	Glycine	3.7 ± 0.5

TABLE II

EFFECT OF METHYLMERCURIC CHLORIDE (CH_3HgCl) ON THE ACTIVE ACCUMULATION OF PUTATIVE NEUROTRANSMITTERS IN RAT GLIOMA CELLS (C-6) IN CULTURE

Cells (10^5) were plated in Lux culture dishes (60 mm), and CH_3HgCl was added 24 h later. Drug and medium were changed 2 days after treatment and the uptake study was performed 3 days after treatment. Experiments were repeated three times involving three dishes per putative neurotransmitter each time. Data are representative of one such experiment.

Treatment	Neurotransmitters	pmoles accumulated/ min/100 mg protein
Control	Choline	0
CH_3HgCl (0.3 μM)	Choline	0
Control	Glutamate	1.2 ± 0.2
CH_3HgCl (0.3 μM)	Glutamate	0.9 ± 0.1
Control	Glycine	1.4 ± 0.3
CH_3HgCl (0.3 μM)	Glycine	0 —

glycine or glutamate increased in NB cells after treatment with CH_3HgCl (1 μM), whereas it decreased in CH_3HgCl-treated (0.3 μM) glioma cells.

Mercury concentrations in serum below 0.5 μM have been regarded as safe [22]. In vitro concentrations of 0.3 μM—1.0 μM CH_3HgCl produce disturbances in high affinity uptake of certain putative neurotransmitters. Previous short-term studies have shown [9—12] that these concentrations of CH_3HgCl inhibit cell division and increase the intracellular level of cyclic AMP in glioma and NB cells. Long-term treatment of glioma and NB cells with lower concentrations of CH_3HgCl (0.05—0.2 μM) produces marked alteration in gene expression as evidenced by changes in the amount and phosphorylation of specific proteins [12, 13], and reduces the sensitivity of adenylate cyclase to prostaglandin E_1 in glioma cells, but not in NB cells [11].

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