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INHIBITION BY METHYLMERCURIC CHLORIDE OF PROSTAGLANDIN E_1-SENSITIVE ADENYLATE CYCLASE ACTIVITY IN GLIOMA BUT NOT IN NEUROBLASTOMA CELLS IN CULTURE

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Abstract—The responses of prostaglandin E_1 (PGE_1)-sensitive adenylate cyclase activity in glioma (C-6) and neuroblastoma (NBP_2) cells in culture after acute (5 days) and chronic (6–7 weeks) treatment with low concentrations (0.1 μM) of methylmercuric chloride (CH_3HgCl) were investigated. The sensitivity of adenylate cyclase activity to PGE_1, as measured by the rise in the intracellular level of cyclic AMP, was markedly reduced in chronically treated glioma cells, but it was not affected in chronically treated NB cells. Acute treatments of glioma and NB cells with CH_3HgCl did not modify the effect of PGE_1 on the intracellular level of cyclic AMP. The responses of dopamine- and norepinephrine-sensitive adenylate cyclases in actuely treated neuroblastoma ($NBA_{2(1)}$) cells and norepinephrine-sensitive adenylate cyclase in acutely or chronically treated glioma cells did not change.

It is well established that methylmercuric chloride (CH_3HgCl) causes a neurological disorder which is referred to as Minamata's Disease [1,2]. CH_3HgCl accumulates in the central nervous system in large proportions after ingestion or after intravenous or intraperitoneal administration [3–6]. To understand the cellular and molecular mechanisms of CH_3HgCl-induced damage to tissue of the nervous system, we used monolayer cultures of rat glioma and mouse neuroblastoma (NB) cells as experimental models. Our previous studies have shown that (a) glioma cells are more sensitive than NB cells to CH_3HgCl using criteria of cell death and inhibition of cell division [7]; (b) the intracellular levels of cyclic AMP increase after treatment of glioma cells (0.3 μM) and NB cells (1 μM) with CH_3HgCl [8]; and (c) chronic treatment of glioma cells with low concentrations (0.05 to 0.1 μM) of CH_3HgCl produces alterations in gene expression, as evidenced by changes in the amounts and phosphorylations of specific proteins [9]. The response of PGE_1-sensitive adenylate cyclase activity of the central nervous system tissue to CH_3HgCl had not been investigated. We now report that the sensitivity of adenylate cyclase activity to PGE_1 was markedly reduced in glioma cells treated chronically with low concentrations of CH_3HgCl, but that no such effect occurred in chronically treated NB cells.

MATERIALS AND METHODS

Mouse neuroblastoma clone (NBP_2) [10], which has both tyrosine hydroxylase and choline acetyltransferase, and clone ($NBA_{2(1)}$) [10], which contains tyrosine hydroxylase but no choline acetyltransferase, were used in this study. Rat glioma cells (C-6) [11] of passages 30–36 [12] were used in this study. Neuroblastoma cells were grown in F12 medium containing 10 per cent agammaglobulin newborn calf serum, whereas glioma cells were grown in F12 containing 10 per cent fetal calf serum. Both types of media contained penicillin (100 units/ml) and streptomycin (100 μg/ml). Cells were maintained at 37° in a humidified atmosphere of 5 per cent CO_2. For chronic CH_3HgCl treatment, cells were grown in 75 cm^2 flasks for 6–8 weeks in the continuous presence of CH_3HgCl. Glioma cells were treated with 0.05 and 0.1 μM CH_3HgCl, whereas NB cells were treated with 0.1 and 0.2 μM. Cells were subcultured every 5 days. CH_3HgCl treatment routinely started 24 hr after plating, because the cells were in the exponential phase of growth at this time. However, in chronically treated cells which required replating every 5 days for a period of 6–7 weeks, it was necessary to add CH_3HgCl at the time of replating for the experiment, beacuse CH_3HgCl-induced damages may recover if CH_3HgCl is not present in the culture for any time interval between addition of CH_3HgCl and experimentation. Acute treatment did not require replating of the cells; therefore, it was not necessary to add CH_3HgCl at the time of plating. The medium and drug were changed 3 and 4 days after plating in both acute and chronic treatments. The effect of PGE_1, norepinephrine and dopamine on the intracellular level of cyclic AMP was measured 5 days after plating. Although dopamine and norepinephrine-sensitive adenylate cyclases are demonstrable [13] in homogenates of neuroblastoma clone $NBA_{2(1)}$, they do not increase the cyclic AMP level until the activity of cyclic AMP phosphodiesterase is inhibited [14]. Therefore, the effect of dopamine and norepinephrine on the intracellular level of cyclic AMP was measured in the presence of 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (R020-1724), a cyclic nucleotide phosphodiesterase inhibitor [15]. Norepinephrine increases the intracellular level of cyclic AMP in glioma cells in the

Table 1. Effects of methylmercuric chloride (CH_3HgCl) on prostaglandin E_1-sensitive adenylate cyclase in glioma and neuroblastoma cells in culture*

Treatment	Cyclic AMP level (pmoles/mg protein) Glioma (C-6)	Neuroblastoma (P_2)
Control	21 ± 2*	20 ± 3
PGE_1 (10 μg/ml)	53 ± 6	36 ± 5
CH_3HgCl, 0.1 μM (acute)	30 ± 3	22 ± 5
PGE_1 + CH_3HgCl (acute)	51 ± 7	36 ± 4
CH_3HgCl, 0.1 μM (chronic)	20 ± 5	20 ± 4
PGE_1 + CH_3HgCl (chronic)	29 ± 4	36 ± 5

* Plating densities were: for NB cells, 0.5×10^5 in control, PGE_1, and 0.1 μM CH_3HgCl groups, and 1×10^5 in the 0.2 μM CH_3HgCl group, for glioma cells, 1×10^5 in control, PGE_1, and 0.05 μM CH_3HgCl groups, and 2×10^5 in the 0.1 μM CH_3HgCl group. Cells were treated with CH_3HgCl either immediately after plating (chronically treated cells) or 1 day after plating (acutely treated cells). After 5 days in culture, fresh growth medium and CH_3HgCl were changed 30 min before the addition of PGE_1. The cells were incubated in the presence of PGE_1 for 10 min and then harvested for cyclic AMP assay. Each value is the mean of eight samples ± standard error of the mean. The values of cyclic AMP levels in control and prostaglandin E_1 (PGE_1)-treated glioma and NB cells were different at $P = 0.001$. The values of cyclic AMP levels in control glioma cells after treatment with PGE_1 and in chronically treated glioma cells after treatment with PGE_1 were different at $P = 0.058$.

absence of an inhibitor of cyclic nucleotide phosphodiesterase [16]. PGE_1 and R020-1724 were dissolved in 50 per cent ethyl-alcohol, whereas dopamine and norepinephrine were dissolved in ascorbic acid solution (1 mg/ml). Dopamine and norepinephrine are autoxidized in solution; therefore, it was necessary to dissolve these neurotransmitters in ascorbic acid solution. Fresh growth medium and CH_3HgCl were added 30 min prior to the addition of cyclic AMP-stimulating agents (PGE_1-10 μg/ml; *l*-dopamine, 10 μM; *l*-norepinephrine, 10 μM). Cells were incubated in the presence of drugs for 10 min. An equivalent volume of ethanol (final concentration, 0.5 per cent) or an equivalent amount of ascorbic acid (final concentration, 10 μg/ml) was added to another set of cultures as a control. The level of cyclic AMP was determined according to the method of Gilman [17], and protein was determined according to the method of Lowry *et al.* [18].

RESULTS AND DISCUSSION

PGE_1 significantly increased the intracellular level of cyclic AMP in both glioma and NB cells ($P = 0.001$, 3-way analysis of variance) 10 min after treatment; however, this effect of PGE_1 was markedly reduced ($P = 0.058$) in chronically treated (0.1 μM) glioma cells, but not in chronically treated (0.1 and 0.2 μM) NB cells (Table 1). Chronic CH_3HgCl treatment at 0.05 μM in the glioma cells also reduced the PGE_1 response, but to a lesser extent than treatment at 0.1 μM. The addition of an equivalent volume of ethanol did not affect the level of cyclic AMP in control or chronically treated cells. The response of PGE_1-sensitive adenylate cyclase did not change after acute treatment with methylmercuric chloride in either glioma or NB cells.

Dopamine (10 μM) and norepinephrine (10 μM) in the presence of R020-1724 (a cyclic nucleotide phosphodiesterase inhibitor) increased the intracellular level of cyclic AMP by about 2-fold more than that produced by R020-1724. R020-1724 by itself increased the cellular cyclic AMP by about 3 to 4-fold. The responses of dopamine- and norepinephrine-sensitive adenylate cyclases in neuroblastoma cells ($NBA_{2(1)}$) did not change after treatment with 0.5 μM CH_3HgCl (data not shown). Norepinephrine (10 μM) by itself increased the intracellular level of cyclic AMP in glioma cells by about 8- to 10-fold, and this effect of norepinephrine remained unaltered in acutely and chronically treated glioma cells (data not shown).

The reasons for a decreased response of PGE_1-sensitive adenylate cyclase in chronically treated glioma cells are unknown. The following possibilities can be mentioned: (a) chronic treatment of glioma cells with CH_3HgCl may cause an increase in cyclic AMP phosphodiesterase activity, which may become the limiting factor in the accumulation of cyclic AMP after treatment of cells with PGE_1; (b) the number of PGE_1 receptors may be decreased after chronic treatment with CH_3HgCl; and (c) a modification of the plasma cell membrane may result in a lessened affinity of PGE_1 to the cyclase receptors. The present data cannot be extrapolated to an *in vivo* condition. However, one can question whether one of the biochemical lesions of CH_3HgCl-induced damage to nervous tissue might not be a reduction in the sensitivity of adenylate cyclase to PGE_1 in glial cells. The dopamine- and norepinephrine-sensitive cyclases were unaffected by acute or chronic treatment of cells with CH_3HgCl.

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