Low-Level Exposure to Methylmercury Modifies Muscarinic Cholinergic Receptor Binding Characteristics in Rat Brain and Lymphocytes: Physiologic Implications and New Opportunities in Biologic Monitoring

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Methylmercury (MeHg) affects several parameters of cholinergic function. These alterations are thought to play a role in MeHg neurotoxicity. *In vitro* experiments have indicated that MeHg acts as a strong competitive inhibitor of radioligand binding to muscarinic cholinergic receptors (mAChRs) in rat brain. Furthermore, rat brain mAChRs share several pharmacologic characteristics of similar receptors present on lymphocytes. Using the muscarinic antagonist [^{3}H]quinuclidinyl benzilate (QNB) to label receptors, we investigated the *in vivo* interactions of MeHg with rat brain mAChRs. We also investigated whether MeHg-induced central mAChR changes are reflected by similar alterations in splenic lymphocytes. Exposure to low doses of MeHg—0.5 or 2 mg/kg/day in drinking water—for 16 days significantly increased (20–44% of control) mAChRs density (B_{max}) in the hippocampus and cerebellum without affecting receptor affinity (K_d). The effect of MeHg did not occur immediately; it was not apparent until 2 weeks after the termination of treatment. No significant changes in [^{3}H]QNB binding were observed in the cerebral cortex. In splenic lymphocytes, mAChR density was remarkably increased (95–198% of control) by day 14 of MeHg exposure and remained enhanced 14 days after the cessation of treatment. These results suggest up-regulation of mAChRs in selected brain regions (hippocampus and cerebellum) after prolonged low-level ingestion of MeHg in rats. These cerebral effects are delayed in onset and are preceded by a marked increase in density of mAChRs on lymphocytes. In chronic MeHg exposure, peripheral lymphocytes may represent a sensitive target for the interaction of MeHg with mAChRs and, therefore, may be predictive indicators of later adaptive response involving cerebral mAChRs. Additionally, the effect of MeHg on lymphocyte mAChRs *in vivo* indicates that this receptor system should be investigated further as a possible target for MeHg immunotoxicity. *Key words*: biomarker, brain, cholinergic system, lymphocyte, methylmercury, muscarinic receptor, neurotoxicity. *Environ Health Perspect* 108:29–33 (2000). [Online 2 December 1999]
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Methylmercury (MeHg) is a ubiquitous and hazardous environmental contaminant. Catastrophic episodes of MeHg poisoning due to accidents or to the consumption of food contaminated by high concentrations of MeHg have been described (*1,2*). Because ingested MeHg is almost completely absorbed from the digestive tract and easily accumulates in the brain, chronic low-level dietary intake of MeHg also poses a significant toxicologic problem. Excessive MeHg intake has been reported in fish-eating communities in Greenland, the Faroe Islands, the Seychelles, the Madeira Basin (in the Amazon region), and New Zealand (*3–5*). There is concern about the risks to human nervous system development associated with such exposures. Several observations indicate that the immature brain is highly susceptible to MeHg toxicity (*6*). In prenatal exposure, deficits in infant brain development have been associated with MeHg levels that cause few, if any, symptoms or signs of toxicity in adults (*7*).

Research data indicate that the cholinergic system is one of multiple targets for MeHg neurotoxicity. *In vitro* MeHg inhibits choline uptake and choline acetyltransferase (ChAT) activity while exerting both stimulatory and inhibitory effects on acetylcholine (ACh) release from central (*8*) and peripheral (*9*) nerve endings. Additionally, MeHg inhibits radioligand binding to brain cholinergic (nicotinic and muscarinic) receptors in a competitive manner (*10–12*). The results of *in vivo* studies support these observations. Long-term administration of MeHg slightly but uniformly decreases cerebral ChAT activity (*13*). As a result, the rate of ACh synthesis and the levels of regional ACh are decreased (*14,15*). In rats, prenatal exposure to MeHg reduced muscarinic binding in the developing brain (*16*).

Radioligand binding studies have also documented the presence of cholinergic muscarinic receptors (mAChRs) in nonexcitable tissues, for example, splenic or circulating lymphocytes, from both rats (*17,18*) and humans (*19,20*). Rat lymphocyte mAChRs share several pharmacologic characteristics of similar receptors present in brain tissue (*21*). Muscarinic binding was modulated by cholinergic and anticholinergic compounds in a similar manner in brain and lymphocytes (*22*). In comparative assays using [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) to label mAChRs, a 14-day exposure to the organophosphate pesticide disulfoton (an acetylcholinesterase inhibitor) caused a significant decrease (25–35%) in mAChRs density in brain areas (cerebral cortex, hippocampus, striatum, and medulla pons), as well as in lymphocytes (*23*). During disulfoton exposure, the decline of mAChR density in lymphocytes paralleled those observed in brain (*23*). These findings suggest that lymphocytes may serve as peripheral markers of the central mAChR changes induced by xenobiotics. Application of mAChR assays using peripheral blood lymphocytes has recently been described in patients with disease states that are likely associated with alteration of the central cholinergic receptors (*24,25*).

There is growing evidence from animal and cell culture studies that MeHg may adversely affect the immune system (*26*). Low concentrations of MeHg (0.6–5 μM) cause dose-dependent changes and cell death in human T cells (*26*). These effects are apparently induced by MeHg by apoptotic mechanisms that also appear to play a major role in MeHg neurotoxicity (*27*).

This study investigated the effects of prolonged low-dose MeHg exposure on the mAChRs present in rats in three brain regions (cerebral cortex, hippocampus, and cerebellum) and on splenic lymphocytes. The discussion relates the results to neurotoxic and immunotoxic effects of MeHg exposure, and to the prospect of using lymphocyte

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mAChRs as surrogate indicators of mAChR changes induced by MeHg in brain tissue.

Materials and Methods

Chemicals. [^{3}H]QNB (49 Ci/mmol) and scintillation fluid Biofluor were obtained from NEN Life Sciences Products (Boston, MA). Methylmercury (II) hydroxide was obtained from Alfa (Karlsruhe, Germany), and all other chemicals were from Sigma Chimica (Milan, Italy).

Animals and treatment. Adult female Sprague-Dawley rats (200–250 g) were obtained from Charles River (Calco, Italy). Animals were randomized to treatment groups and housed one per cage under constant conditions of temperature, humidity, and photocycle (12-hr light/12-hr dark) for at least 1 week before study. A commercial rat diet (4RF21; Mucedola, Settimo Milanese, Italy) and water were given *ad libitum.*

Rats were given MeHg for 16 consecutive days. Three groups of animals received MeHg in their drinking water at nominal concentrations of 0, 2.5, and 10 µg/mL, respectively. Fresh MeHg solutions were prepared daily from a 1 M stock solution of methylmercury (II) hydroxide in water. Water consumption and body weight were recorded daily during the study. MeHg intake in the two groups of MeHg-exposed rats averaged (mean ± SD) 0.45 ± 0.052 and 1.8 ± 0.212 mg/kg/day, respectively. Rats were sacrificed by decapitation either immediately or 14 days after the termination of MeHg treatment. The spleens and brains were rapidly removed and placed in 32 mL Hank's buffer (without calcium and magnesium; pH 7.5) on ice. Three brain areas (cerebral cortex, cerebellum, and hippocampus) were dissected, weighed, and then stored at -80°C until receptor binding assays were performed.

All experiments complied with European Council Directive 86/609/EEC on the care and use of laboratory animals (*28*).

Isolation of splenic lymphocytes. The spleen was teased apart with forceps in Hank's buffer, and 8 mL cell suspension was layered on 3 mL Histopaque 1,083 (Sigma Chimica, Milan, Italy) according to the method of Boyum (*29*). After centrifugation at 400*g* for 30 min, the layer containing lymphocytes was transferred to another plastic tube, resuspended in Hank's buffer, and washed three times by centrifugation (200*g* for 10 min). The cells were resuspended in the same buffer and counted on a Coulter counter (Instrumentation Laboratory, Monza, Italy).

Preparation of brain tissue membranes. Membranes were prepared by homogenization with Polytron (Braun, Germany) for 20 sec. Brain areas were homogenized in 20 volumes ice-cold 0.05 M Na/K phosphate buffer (pH 7.4). The homogenate was centrifuged three times at 49,000*g* for 10 min at 4°C.

[^{3}H]QNB binding in lymphocytes. Saturation binding of [^{3}H]QNB to lymphocytes was performed as described by Fitzgerald and Costa (*23*). Briefly, 2 million lymphocytes were incubated in a range of [^{3}H]QNB concentrations (7.5–240 nM) and Hank's buffer in a total volume of 0.5 mL in plastic tubes. After 60 min incubation under gentle shaking at 27°C, the reaction was stopped by adding 3 mL ice-cold phosphate-buffered saline (PBS); samples were rapidly filtered through Whatman GF/C filters (Whatman, Maidstone, Kent, UK) using a Brandel cell harvester (Emanuele Mires, Milan, Italy) and washed three times with 3 mL ice-cold PBS. Filters were then air dried and counted for radioactivity in 3 mL Biofluor in a Packard 1900 CA scintillation counter (Packard, Milan, Italy). Atropine (10^{-4} M) was added to half of the tubes for estimation of specific binding, defined as the difference between binding in the absence and the presence of atropine. In a series of preliminary experiments, the influence of different incubation times and temperatures on [^{3}H]QNB binding was tested. Each sample was assayed in triplicate and data were expressed as fmol/10^6 cells.

[^{3}H]QNB binding in brain membranes. Saturation [^{3}H]QNB binding was performed in membrane preparations from cerebral cortex, cerebellum, and hippocampus by the method of Yamamura and Snyder (*30*), as previously described (*22,31*). Atropine (10^{-5} M) was added 10 min before [^{3}H]QNB to half of the tubes for estimation of nonspecific binding. Samples were incubated with [^{3}H]QNB concentrations ranging from 0.025 to 1 nM. Binding assay was carried out as described for lymphocytes. Protein content was determined by the method of Lowry et al. (*32*). Data were expressed as femtomoles per milligram of protein.

Analysis of data. Receptor density (B_{max}) and affinity (defined as the reciprocal of the dissociation constant, K_d) were estimated by nonlinear regression analysis of binding data according to the methods described by Bylund and Yamamura (*33*). The Hill coefficients (n_H) were derived from

$$\log[B/(B_{max} - B)] = n_H \times \log F - \log K_d,$$

where B is the concentration of bound ligand at any given concentration of free ligand (F) (*33*). Analysis of variance for continuous-by-class effects was used to test statistical differences in saturation curve profiles between MeHg-treated and control rats. Statistical analysis was performed using the SAS system

Table 1. Muscarinic receptor binding in MeHg-treated rats: end of treatment.[a]

	Cerebral cortex (mean ± SE)	Hippocampus (mean ± SE)	Cerebellum (mean ± SE)	Lymphocytes (mean ± SE)
Control				
B_{max}	2,016 ± 204	1,505 ± 213	157 ± 13	87 ± 33
K_d	0.34 ± 0.01	0.19 ± 0.03	0.05 ± 0.01	67 ± 17
n_H	1.13 ± 0.02	1.12 ± 0.03	0.95 ± 0.03	0.98 ± 0.01
MeHg (0.5 mg/kg/day)				
B_{max}	2,296 ± 90	1,623 ± 262	167 ± 21	170* ± 34
K_d	0.37 ± 0.04	0.19 ± 0.01	0.05 ± 0.01	63 ± 9
n_H	1.11 ± 0.01	1.06 ± 0.03	0.96 ± 0.01	0.99 ± 0.06
MeHg (2 mg/kg/day)				
B_{max}	2,066 ± 162	1,679 ± 228	162 ± 33	259* ± 58
K_d	0.32 ± 0.04	0.19 ± 0.02	0.05 ± 0.01	80 ± 10
n_H	1.11 ± 0.01	1.10 ± 0.02	0.93 ± 0.01	0.92 ± 0.02

Abbreviations: B_{max}, receptor density expressed as fmol/mg protein (brain) or fmol/10^6 cells (lymphocytes); K_d, receptor affinity (nM); n_H = Hill coefficient.
[a]Rats were treated with MeHg in drinking water for 16 days and sacrificed immediately after termination of exposure. Values are the mean ± SE of three separate experiments in which tissues from four rats were pooled. *$p < 0.05$, statistically different within a single experiment comparing the [^{3}H]QNB saturation curve from MeHg-treated rats with its concurrent control curve.

Table 2. Muscarinic receptor binding in rats 14 days after termination of treatment with MeHg.[a]

	Cerebral cortex (mean)	Hippocampus (mean)	Cerebellum (mean)	Lymphocytes (mean)
Control				
B_{max}	1,868	1,345	145	110
K_d	0.47	0.30	0.05	55
n_H	1.09	1.12	0.89	1.02
MeHg (0.5 mg/kg/day)				
B_{max}	2,077	1,418	174*	190*
K_d	0.53	0.34	0.05	49
n_H	1.09	1.11	0.93	0.94
MeHg (2 mg/kg/day)				
B_{max}	1,969	1,934*	184*	274*
K_d	0.36	0.37	0.05	50
n_H	1.11	1.11	0.91	1.14

Abbreviations: B_{max}, receptor density expressed as fmol/mg protein (brain) or fmol/10^6 cells (lymphocytes); K_d, receptor affinity (nM); n_H = Hill coefficient.
[a]Values are the mean of two separate experiments in which tissues from four rats were pooled. *$p < 0.05$, statistically different within a single experiment comparing the [^{3}H]QNB saturation curve from MeHg-treated rats with its concurrent control curve.

computer program (SAS Institute, Inc., Cary, NC).

Results

Effects of exposure. Rats given 0.5 or 2.0 mg/kg/day MeHg in their drinking water remained physically normal and were behaviorally similar to controls. Daily water consumption and body weight gain were similar in controls and in MeHg-treated animals (data not shown). The absorption of mercury was not measured. In separate experiments on pregnant rats given 0.5 mg/kg MeHg in the drinking water from day 7 of gestation until day 7 of lactation, we measured total mercury concentrations of approximately 1 µg/g in the brain and cerebellum of the adult animals (*34*).

[^{3}H]QNB binding in brain. The experiments performed immediately after the 16-day exposure period showed no significant differences in [^{3}H]QNB binding between control and MeHg-treated rats in any of the brain regions (cerebral cortex, hippocampus, and cerebellum) examined (Table 1). However, binding was significantly increased in the hippocampus and cerebellum of the MeHg-exposed animals allowed to survive 14 days after termination of treatment (Table 2). Data from representative experiments illustrating the delayed effect of MeHg on receptor binding in the cerebellum are shown in Figure 1. In the postexposure period, cerebellar mAChRs density (B_{max}) was increased by 20 and 27% in rats treated with 0.5 and 2 mg/kg MeHg, respectively. In hippocampus, a 44% increase in the B_{max} was observed in rats in the 2 mg/kg group, whereas no significant changes in [^{3}H]QNB binding were seen in the animals given the lower (0.5 mg/kg) MeHg dose. Muscarinic binding was not affected in the cerebral cortex at any of the MeHg doses tested (Tables 1 and 2).

In all brain areas, MeHg did not affect the affinity of mAChRs, as indicated by the dissociation constant values (K_d) measured either immediately or 14 days after termination of exposure (Tables 1 and 2). The Hill coefficients (n_H) were close to unit (0.89–1.13) in both control and MeHg-treated rats, suggesting homogeneous affinity of the labeled receptors for the ligand.

[^{3}H]QNB binding in splenic lymphocytes. In rats given MeHg, significant increases in splenic lymphocyte [^{3}H]QNB binding were observed both immediately after exposure (Table 1) and 14 days after the cessation of treatment (Table 2). The effects of MeHg were dose dependent. Lymphocyte mAChRs receptor density (B_{max}) increased 73–95% after treatment with 0.5 mg/kg/day and 150–198% after treatment with 2 mg/kg/day.

In contrast with the effect on receptor binding in brain tissue, the changes induced by MeHg in lymphocytes were present immediately at the end of the exposure period and were still observable 14 days after termination of treatment (Figure 2). Analyses of data from saturation curves of [^{3}H]QNB binding (Figure 3) clearly illustrate the different pattern and regional selectivity of the response to MeHg with changes in receptor density that developed earlier and were more pronounced in lymphocytes than in brain tissue.

As in the brain, the affinity of lymphocyte mAChRs (K_d) was not affected by MeHg treatment, and the Hill coefficients (n_H) were close to unit (0.92–1.14) in both control and MeHg-treated rats (Tables 1 and 2).

Discussion

In this study, prolonged exposure of rats to MeHg at a daily dose as low as 0.5 mg/kg caused molecular changes involving

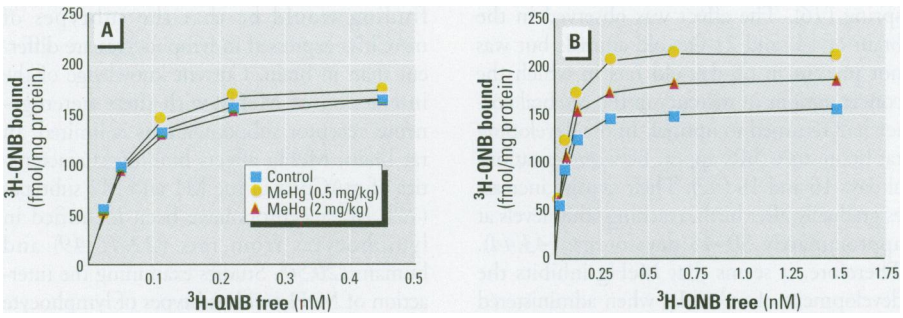

Figure 1. Binding characteristics of muscarinic receptor in the cerebellum of rats treated with MeHg. Saturation curves of [^{3}H]QNB binding in animals sacrificed (*A*) immediately at the end of a 16-day exposure period or (*B*) 14 days after termination of treatment. Tissue preparations were incubated with increasing concentrations of the radioligand alone (total binding) or plus atropine (10^{-5} M) to define nonspecific binding. Specific binding was obtained by subtracting nonspecific from total binding. The different curve profiles in (*A*) and (*B*) highlight the delayed effect of MeHg exposure on cerebellar receptor binding. Postexposure data (*B*) shows that curve profiles for MeHg-treated rats are different ($p < 0.05$) from the control curve. Values are the means of triplicate determinations from a representative experiment. Standard errors < 5%.

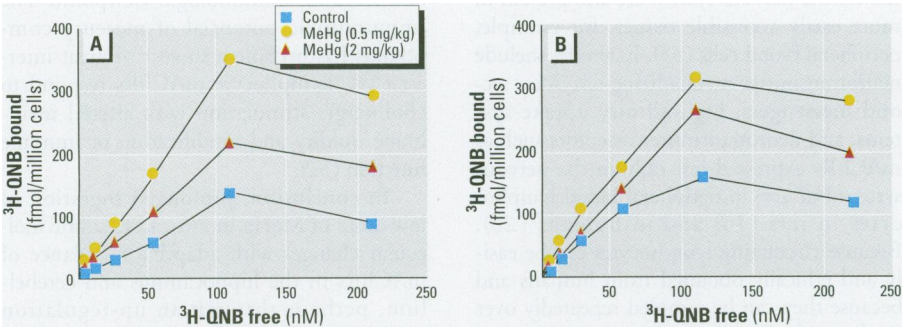

Figure 2. Muscarinic receptor binding in splenic lymphocytes after treatment with MeHg. Saturation curves of [^{3}H]QNB binding in animals (*A*) sacrificed immediately at the end of a 16-day exposure period or (*B*) 14 days after termination of treatment. Curve profiles for MeHg-treated rats are different ($p < 0.05$) from the control curve. Values are the means of triplicate determinations from a representative experiment. Standard errors < 10%.

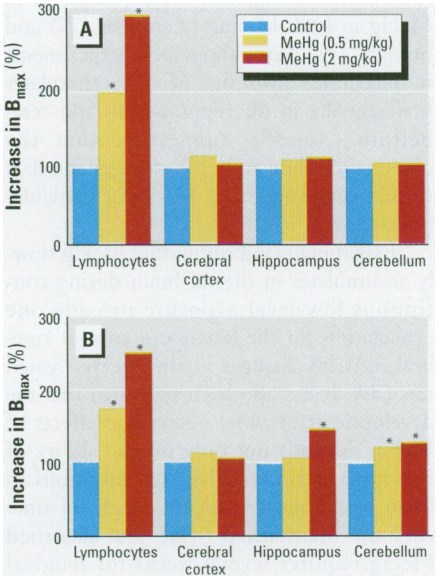

Figure 3. Percentage increase in B_{max} (*A*) at the end of the 16-day MeHg treatment (values are the mean derived from three different experiments) and (*B*) at 14 days after termination of MeHg exposure (values are the mean derived from two different experiments). B_{max} is expressed as fmol/mg protein (brain) or fmol/10^6 cells (lymphocytes).
*$p < 0.05$, statistically different within a single experiment comparing the [^{3}H]QNB saturation curve from MeHg-treated rats with its concurrent control curve.

cholinergic receptors in brain and lymphocytes. These findings are discussed in relation to the effects of MeHg on the nervous system and in relation to perspectives in the area of markers of subclinical MeHg toxicity.

The cholinergic system and MeHg neurotoxicity. MeHg ingestion caused selective increases in mAChR density in two rat brain regions (hippocampus and cerebellum) without affecting receptor affinity. These findings agree with earlier *in vitro* studies describing the alteration of muscarinic receptor binding characteristics in rat brain preparations treated with MeHg (*12*). The MeHg action on cerebral mAChRs was delayed in onset. Increase in receptor density was not demonstrable immediately after termination of exposure but was invariably seen in the animals examined 2 weeks after the end of exposure.

Because cholinergic systems play an important role in learning and memory processes (*35*), the increased mAChR density caused by MeHg in brain tissue may be a deleterious process. The MeHg-induced changes in cerebral mAChR density were regionally specific. The cerebellum, a primary target for MeHg neurotoxicity, was the most susceptible area showing delayed receptor changes even at the lower 0.5 mg/kg/day MeHg dose. Hippocampal mAChR density was increased 14 days after exposure to 2 mg/kg/day MeHg, whereas cerebrocortical receptors were not affected at either dosing regimen. Several studies report selective or focal brain damage as a typical response to MeHg in both laboratory animals (*27*) and humans (*1*). Autoradiographic experiments in mice have shown that MeHg accumulates preferentially in the hippocampus and cerebellum (*36–38*), suggesting that the observed cerebral mAChR changes correlate to the concentration of MeHg in particular brain regions.

Experiments indicating that MeHg slowly accumulates in the rat brain during continuous low-level exposure provide one explanation for the late appearance of cerebral mAChR changes in the MeHg-treated rats (*39*). It has also been suggested that the development of some neurologic effects of MeHg depends not only on the ability of MeHg to attain effective concentrations in brain tissue but also on the length of time they are maintained (*40*). The absorbed MeHg requires several weeks for removal from the rat brain (*16,39*). Adaptive changes involving mAChRs in the target organ may occur after prolonged overexposure to MeHg. Neurotransmitter receptors are regulated by homeostatic mechanisms that compensate for changes in the amount of agonist or antagonist to which they are exposed (*22,23*). For example, although mAChRs overstimulation induces a receptor density decrease (down-regulation), prolonged treatments with muscarinic antagonists (e.g., atropine) cause receptor increase (up-regulation) and supersensitivity to muscarinic agonists (*22*). Thus, the delayed effect of MeHg in the rat brain might be attributable to a combination of factors. Gradual accumulation of MeHg in brain tissue and up-regulation of cerebral mAChRs may occur during chronic MeHg exposure to compensate for early stage effects such as *a*) MeHg-induced inhibition of ACh synthesis (*13*) and consequent reduction of brain ACh levels (*14,15*), and *b*) direct (atropinelike) competitive antagonism by MeHg on mAChRs (*10–12*). It is increasingly evident that adaptation of neurotransmitter receptors may be a primary mechanism mediating the long-term actions of drugs and chemicals in the central nervous system (*41*).

Our findings in adult rats differ from observations in the immature rat brain where MeHg decreased and did not increase [^{3}H]QNB binding (*16*). In pregnant rats, a single dose of 8 mg/kg MeHg by gavage on day 15 of gestation temporarily reduced the density of cerebrocortical mAChRs in the offspring (*16*). The effect was observed in the brain of 14- and 21-day-old animals but was not present in 60-day-old rats in which the concentrations of mercury in the cerebral cortex had returned to normal. In the developing rat brain, mAChRs appear between gestational days 16 and 18 (*42*). Their density increases gradually after birth, reaching adult levels at approximately 30–45 days of age (*43,44*). Therefore, it seems that MeHg inhibits the development of mAChRs when administered before they appear. Perhaps the postulated up-regulation mechanism, which in the adult compensates for the complex interactions of MeHg with the cholinergic system, is not functional in the immature brain.

Muscarinic cholinergic receptors as biomarkers. It is increasingly evident that biochemical and molecular parameters similar to those involved as targets for toxicants in the central nervous system are also present in more easily accessible tissues, for example, peripheral blood cells (*45*). Examples include neurotransmitter metabolizing enzymes, second messengers, high-affinity uptake systems, and neurotransmitter receptors such as mAChRs expressed not only in the nervous system but also in peripheral blood lymphocytes in rats (*18*) and in humans (*20*). Because circulating lymphocytes can be easily and ethically obtained from humans and because they can be sampled repeatedly over prolonged chemical exposure, strategies using these peripheral samples as surrogate indicators offer the advantage of exploring early effects of neurotoxicants by convenient noninvasive methods (*46*).

In rats exposed to MeHg, significant increases in mAChR density were found not only in hippocampus and cerebellum but also in splenic lymphocytes. The receptor increase in lymphocytes was even more pronounced (up to 198% vs. 20–44%) and more rapid in onset than that in brain tissue. There was no temporal correlation for the receptor changes occurring in MeHg response in brain and lymphocytes. The reason for this discrepancy is not evident. In rats, prolonged low-level administration of MeHg caused higher (up to 10-fold) mercury levels in the spleen than in brain (*47*). In chronic exposure, the effective MeHg concentration required to modify mAChRs density may be more rapidly attained in peripheral lymphocytes than in the less accessible brain tissue where the rates of access and accumulation of MeHg are limited by the blood–brain barrier.

Muscarinic receptors belong to a multigenic family that codes for at least five receptor subtypes (*48*). At control levels, [^{3}H]QNB displayed a lower affinity in lymphocytes than in brain tissue, confirming previous observations (*21*). A possible explanation for this finding would be that the subtypes of mAChRs expressed in lymphocytes are different than in brain. Current knowledge of the interaction of MeHg with these heterogeneous receptor subpopulations is limited. In rat brain, MeHg affects binding characteristics of mAChRs of the M1 and M2 subtypes (*12*). M2 receptors have been identified in lymphocytes from rats (*22,46,49*) and humans (*20,50*). Studies examining the interaction of MeHg with subtypes of lymphocyte muscarinic receptors would be helpful to establish whether the muscarinic cholinergic recognition sites expressed in peripheral tissues are representative of the corresponding molecular parameters present in the central nervous system.

The interaction of MeHg with mAChRs in lymphocytes may have important implications not only in relation to biomarkers but also from an immunologic viewpoint. The immunotoxic potential of mercury compounds has become a subject of great interest (*51*). Lymphocyte mAChRs respond to cholinergic stimulation with altered membrane fluidity and modification of immune function (*52*).

In conclusion, prolonged ingestion of low doses of MeHg in rats causes subtle molecular changes with adaptive imbalance of mAChRs in the hippocampus and cerebellum, perhaps through an up-regulation mechanism. The onset of the effect of MeHg is delayed and is preceded by a marked increase in density of mAChRs expressed in splenic lymphocytes. Because it is impossible to routinely assess neurotransmitter receptor

function in the mammalian nervous system *in vivo*, mAChRs in peripheral blood lymphocytes may be convenient surrogate indicators of analogous receptor changes occurring in brain after prolonged exposure to MeHg or to other toxicants that affect mAChRs in the central nervous system. Additionally, the effect of low doses of MeHg on lymphocyte mAChRs indicates that this receptor system should be investigated further as a possible target for MeHg immunotoxicity.

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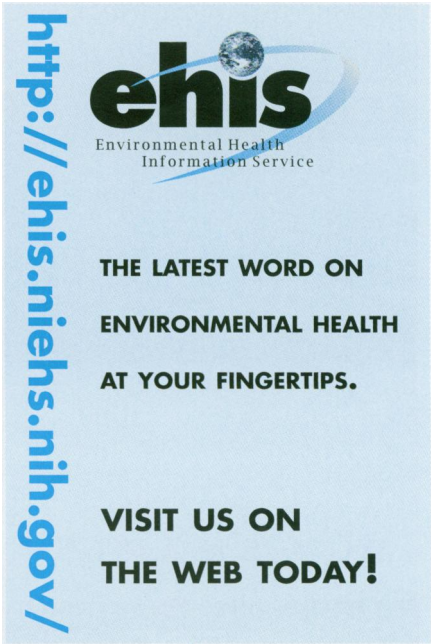
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