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Regeneration of a Functionally Active Rat Brain Muscarinic Receptor by D-Penicillamine after Inhibition with Methylmercury and Mercuric Chloride

Evidence for Essential Sulfhydryl Groups in Muscarinic Receptor Binding Sites

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SUMMARY

The molecular mechanism of methylmercury and mercuric chloride inhibition of brain muscarinic acetylcholine receptor is investigated. Both mercuric cations strongly inhibit L-[^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding to rat brain lysed synaptosomes. Mercuric chloride is 350 times more potent as an inhibitor of [^{3}H]QNB binding than is methylmercury. Inhibition of the agonist binding site by methylmercury is demonstrated by the competitive action of carbamylcholine chloride on [^{3}H]QNB binding. D-Penicillamine is found to chelate mercuric cations from the receptor binding site and regenerate the [^{3}H]QNB binding in a concentration-dependent manner. The tightness of mercuric chloride interaction with the receptor binding site is demonstrated by measuring [^{3}H]QNB binding before and after extensive washing. The correlation between mercuric chloride inhibition and D-penicillamine regeneration of [^{3}H]QNB binding emphasizes the involvement of sulfhydryl groups in muscarinic receptor binding site. These essential sulfhydryl groups may have a significant role in the proper functional configuration of the receptor binding site. Blocking these essential sulfhydryl groups is suggested to be the molecular mechanism of inhibition of brain muscarinic receptors by these mercurials. Through mercuric chloride inhibition and D-penicillamine regeneration of [^{3}H]QNB binding, the mercuric cation apparently stabilizes or protects the binding site (compared with control) while the protein is subjected to experimental protocol. This may provide a basis for using mercuric chloride as a probe to protect the receptor binding site in solubilization, isolation, and purification of muscarinic receptors.

INTRODUCTION

In spite of several documented incidents involving methylmercury poisoning, the molecular mechanism of its action has not been well defined. Behavioral (1), pathological (2, 3), histological (4), and clinical (1) findings in humans and primates following methylmercury exposure indicate structural and functional damage in the central and peripheral nervous systems. Electrophysiological studies predict postsynaptic (5) and presynaptic (6) effects of methylmercury. Biochemically, methylmercury strongly inhibits acetylcholine binding to *Torpedo* nicotinic receptors (7, 8). Recently, results from our laboratory (9) have shown *in vitro* and *in vivo* inhibition of rat brain muscarinic receptors by methyl mercury and mercuric chloride.

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Thiols are known to chelate mercury with different affinities. D-Penicillamine (dimethylcysteine), unlike cysteine, is resistant to desulfhydration and oxidation by cysteine desulfhydrase and L-amino acid oxidase, respectively (10). D-Penicillamine is known to be a nontoxic compound, and most of the DL-penicillamine toxicity is attributable to the L-enantiomer (11). Clinically, D-penicillamine has been used effectively as a drug in the treatment of hepatolenticular degeneration (Wilson's disease) (12), cystinuria and the associated nephrolithiasis (13), and rheumatoid arthritis (14). *N*-Acetyl DL-penicillamine also lowers the concentration of mercury in the blood of experimental animals (15). D-Penicillamine effectively prevents accumulation of methylmercury in the fetal brain (16). Warnick and Albuquerque (17) recently reported that D-penicillamine treatment resulted in improving the ability of dystrophic chickens to right themselves.

The present studies were undertaken to characterize the molecular mechanism of inhibition of rat brain mus-

carinic acetylcholine receptors by methylmercury and mercuric chloride. We investigated the role of the essential sulfhydryl groups in muscarinic receptor binding site with respect to the agonist and antagonist binding and their sensitivity to methylmercury and mercuric chloride. Regeneration of functionally active rat brain muscarinic receptors after inhibition with either methylmercury or mercuric chloride was achieved by D-penicillamine. The basis for possible clinical use of D-penicillamine to prevent neurological damage in cases of methylmercury intoxication is discussed.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (325–350 g) were purchased from Taconic Farm (Germantown, N. Y.). [^{3}H]QNB[1] was obtained from New England Nuclear Corporation (Boston, Mass.) and from Amersham Corporation (Arlington Heights, Ill.). Methylmercury chloride (more than 95% pure) was purchased from Ventron Division of Alfa Products (Danvers, Mass.). The chemical analysis of methylmercury obtained from the company indicated that the major impurity (2–3%) was methylmercuric acetate. Other minor impurities were ammonium sulfate and methyl alcohol. No evidence for any detectable inorganic mercury was mentioned. Mercuric chloride was purchased from Fisher Scientific Company (Pittsburgh, Pa.). Other chemicals and reagents were obtained from Sigma Chemical Company (St. Louis, Mo.).

Preparation of rat brain synaptosome. Animals were killed by decapitation. The whole brain minus brain stem was removed and washed in an ice-cold sucrose solution containing 0.32 M sucrose and 50 mM Tris-acetate (pH 7.4). Brain tissues were homogenized in sucrose solution in an ice-cold Thomas glass homogenizer. The suspension was centrifuged at 3,000 × *g* for 10 min in a Beckman J-21 centrifuge. The supernatant fraction was recentrifuged at 40,000 × *g* for 30 min. The The sediment was suspended in the buffered sucrose solution using a Potter-Elvehjem glass homogenizer with a Teflon pestle. The suspension was further subfractionated using a discontinuous sucrose density gradient consisting of 7 ml of 1.4, 1.2, and 0.8 M sucrose in 32-ml cellulose nitrate tubes. The gradients were centrifuged in a Beckman L8-55 ultracentrifuged for 4 or 18 hr using an SW-27 rotor at 25,000 rpm. Synaptosomal fractions sedimented at the interphase between 0.8 and 1.2 M sucrose were collected and diluted to 0.32 M sucrose and osmotically lysed in ice-cold distilled water (pH was adjusted to 7.4 with Tris) for 15 min at 4°. The suspension was then centrifuged at 100,000 × *g* for 30 min. The pellet was resuspended in 0.32 M sucrose solution buffered with 50 mM Tris-acetate (pH 7.4) to give 1.5–2 mg of protein per milliliter. Protein was determined as described by Lowry *et al.* (18), using bovine serum albumin as the standard protein.

Muscarinic receptor binding assay. Rat brain lysed synaptosomes (150–200 μg of protein) were incubated in a medium (1 ml) containing 50 mM Tris-acetate (pH 7.4) and 2 nM [^{3}H]QNB (40–60 Ci/mmole) for 1 hr at 22° with gentle agitation. Nonspecific (atropine-insensitive) [^{3}H]-QNB binding was determined in the presence of 2×10^{-6} M atropine. Free [^{3}H]QNB was separated from the ligand bound to synaptosomal membranes by filtration with suction through a Whatman GF/B Fiberglas filter. The filter was washed with 10 ml of 50 mM Tris-acetate buffer (pH 7.4). Filters were placed in scintillation vials and mixed with 7 ml of scintillation cocktail. The radioactivity of the filter was counted in a B-Rack LKB liquid scintillation counter with 40% efficiency. Each binding assay was determined in three to five replicates.

Methylmercuric chloride or mercuric chloride was incubated with rat brain lysed synaptosomal membranes (150–200 μg) for 1 hr in an incubation medium (980 μl) containing 50 mM Tris-acetate (pH 7.4) prior the addition of 20 μl of 10^{-7} M [^{3}H]QNB. In regeneration experiments, synaptosomal preparation was incubated first with methylmercury or mercuric chloride for 1 hr, and a certain concentration of D-penicillamine was then added and incubated for 1 more hr before assaying for [^{3}H]QNB binding as mentioned above.

RESULTS

[^{3}H]QNB has been used as an excellent marker for muscarinic acetylcholine receptor binding site (19). Carbamylcholine (a specific agonist) strongly competes with [^{3}H]QNB for the receptor binding site. The inhibition of [^{3}H]QNB binding with varying concentrations of carbamylcholine is saturable (Fig. 1, *inset*). Methylmercury is found to inhibit the competitive action of carbamylcholine on [^{3}H]QNB binding in a concentration-dependent manner (Fig. 1). On the other hand, the inhibition of the antagonist binding to muscarinic receptors by methylmercury and mercuric chloride is demonstrated by the decrease in [^{3}H]QNB binding to rat brain lysed synaptosomal membranes (Fig. 2). It is clear that most of the inhibition (0–100%) of [^{3}H]QNB binding was observed over a small range of concentrations of either methylmercury (3×10^{-6} to 5×10^{-4} moles/mg of protein) or mercuric chloride (3×10^{-8} to 6.3×10^{-7} moles/mg of protein). The concentration of methylmercury at which 50% inhibition (I_{50}) of [^{3}H]QNB binding is about 350 times greater than that of mercuric chloride if the comparison is based on moles of inhibitor per milligram of protein. The I_{50} values would be different if compared only on the basis of their molar concentrations without accounting for the amount of protein in each assay. Early studies (9) have shown that mercuric chloride is about 100 times more inhibitory than is methylmercury on rat brain muscarinic receptor based on the molar ratio of I_{50} values. In the present work, methylmercury was freshly prepared in a dimethyl sulfoxide-ethanol (1:3) mixture before each experiment was conducted.

To investigate the role of sulfhydryl groups in muscarinic receptor binding sites in relation to [^{3}H]QNB binding and their susceptibility to mercurials, we examined the effects of simple monothiols on methylmercury and mercuric choloride inhibition of muscarinic receptors. Although L-cysteine slightly reverses methylmercury inhibition of [^{3}H]QNB binding, it causes by itself considerable inhibition (45%) of the control (Table 1). Unlike cysteine, D-penicillamine has no effect on [^{3}H]QNB bind-

[1] The abbreviations used are: [^{3}H]QNB, L-[^{3}H]quinuclidinyl benzilate; NEM, *N*-ethylmaleimide.

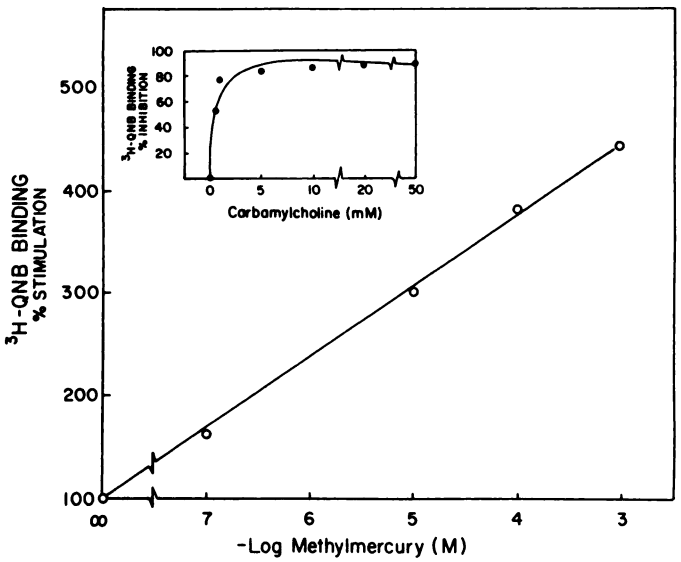

FIG. 1. *Effect of methylmercury chloride on the agonist binding site of rat brain muscarinic receptors*

Rat brain lysed synaptosomal membranes (150–200 μg of protein) were incubated with varying concentrations of methylmercury for 1 hr at 22°. [^{3}H]QNB binding was assayed in the presence of 20 mM carbolamylcholine and 2 nM [^{3}H]QNB. The *inset* shows the effect of carbamylcholine on [^{3}H]QNB binding at the same protein concentration.

ing of the control samples (Figs. 3 and 4). D-Penicillamine successfully reversed the inhibition of muscarinic receptor by methylmercury and mercuric chloride. At a given concentration (5×10^{-5} M) of either inhibitor, reactivation of [^{3}H]QNB binding was consistently dependent on D-penicillamine concentration (Fig. 3). Because of the bifunctionality of mercuric ion, more D-penicillamine is apparently required to reverse the inhibition of muscarinic receptor by mercuric chloride than is required for methylmercury.

Since mercuric chloride was a more potent inhibitor and more readily soluble in aqueous medium than was methylmercury, we examined D-penicillamine regeneratio of [^{3}H]QNB binding at three levels of mercuric chloride (Fig. 4a). The D-Penicillamine concentration required to reactivate muscarinic receptor is very much dependent on the mercuric chloride concentration. At a high mercuric chloride concentration (5×10^{-5} M), D-penicillamine does not completely counteract the mercuric effect. Plotting D-penicillamine concentrations for 50% reversal of [^{3}H]QNB binding inhibition versus mercuric chloride concentrations (Fig. 4b) showed a straight-line function relationship.

In order to examine the tightness of the complex between mercuric ion and the receptor protein in relation to the receptor functional state, i.e., [^{3}H]QNB binding, an extensive washing of the free mercuric or/and D-penicillamine from the incubation medium was performed. Each washing step included the following sequence: (a) dilution of the incubation medium to 5 times the original volume, (b) homogenization using an ice-cold Potter-Elvehjem glass homogenizer fitted with a Teflon

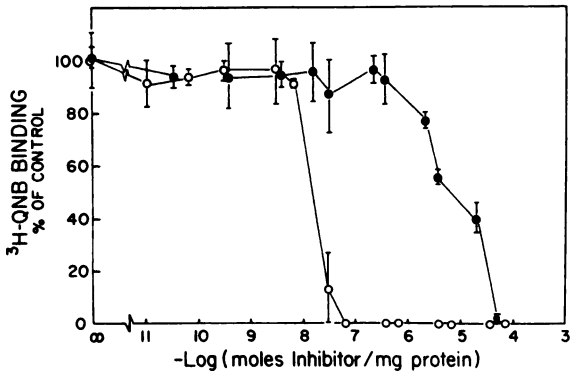

FIG. 2. *Inhibition of [^{3}H]QNB binding to rat brain muscarinic receptors by methymercury (●) and mercuric chloride (○)*

Results were based on milligrams of protein to eliminate variations between separate experiments. Each *point* represents the mean of three to five different experiments ± standard error of the mean.

TABLE 1

Effect of cysteine on rat brain muscarinic receptor inhibition by methylmercury

Treatment	[^{3}H]QNB binding inhibition
	% of control
Cysteine,[a] 5×10^{-3} M	45.0
Methylmercury,[a] 5×10^{-3} M	57.7
Cysteine + methylmercury[b]	47.4
Methylmercury + cysteine[c]	29.3

[a] Rat brain synaptosomal membranes (150–200 μg of protein) were incubated with either cysteine or methylmercury for 1 hr before assaying for [^{3}H]QNB binding.

[b] Rat brain synaptosomal membranes were incubated with cysteine (5×10^{-3} M) for 30 min; methylmercury (5×10^{-3} M) was then added and the incubation was continued for another 30 min before assaying for [^{3}H]QNB binding.

[c] Rat brain synaptsomal membranes were incubated with methylmercury (5×10^{-5} M) for 30 min; cysteine (5×10^{-3} M) was then added and the incubation was continued for another 30 min before assaying for [^{3}H]QNB binding.

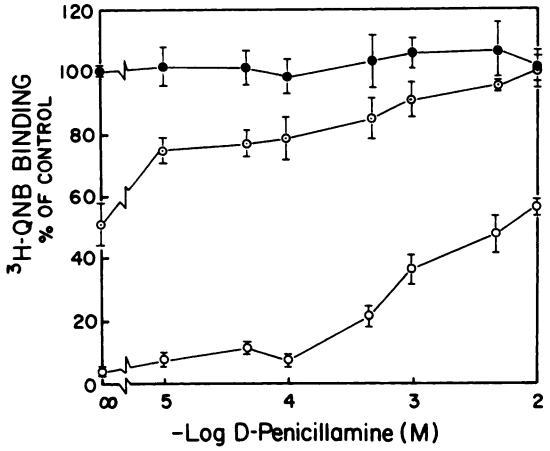

FIG. 3. *Effect of D-penicillamine on rat brain muscarinic receptors in response to 5×16^{-5} M methylmercury (◎) or 5×10^{-5} M mercuric chloride (○) inhibition*

Each *point* represents the mean of three different observations ± standard error of the mean corrected for the corresponding D-penicillamine-treated control (●).

pestle, and (c) centrifugation at 100,000 × *g* for 30 min. The sedimented pellet was resuspended in a solution of 0.32 M sucrose-50 mM Tris-acetate (pH 7.4). Aliquots were taken for the [^{3}H]QNB binding assay and for protein determinations. Previously, Eldefrawi *et al.* (20) demonstrated that [^{14}C]methylmercury is tightly bound to *Torpedo* nicotinic receptor and is not removed by dialysis for 48 hr in a methylmercury-free buffer. In the present work we found that incubation of mercuric chloride (5×10^{-5} M) with rat brain synaptosomes for 1 hr results in about 66% inhibition of [^{3}H]QNB binding of the control (Fig. 5A). Washing the free mercuric ions from the incubation medium did not change the percentage of inhibition of [^{3}H]QNB binding resulting from preincubation with mercuric chloride (Fig. 5B). Incubation of the same protein (Fig. 5B) samples with 5×10^{-3} M D-penicillamine for 1 hr resulted in an appreciable regeneration of [^{3}H]QNB binding to the receptor protein (Fig. 5C). Further washing of the Hg^{2+}-D-penicillamine complex and free D-penicillamine showed a complete regeneration of [^{3}H]QNB binding. It was noted that a series of extensive washing of the preparation resulted in a decrease in the [^{3}H]QNB binding of the control. These results provide evidence for the inhibition and regeneration of a functionally active muscarinic receptor by mercuric chloride and D-penicillamine, respectively.

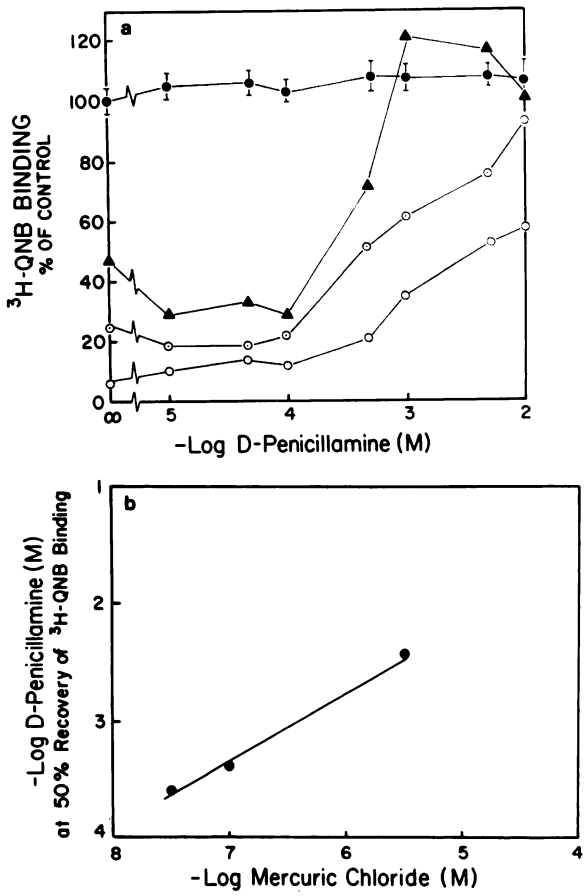

FIG. 4. *Reactivation of rat brain muscarinic receptors*

a. Reactivation of rat brain muscarinic receptors after inhibition with 5×10^{-8} M (▲), 1×10^{-7} M (⊙), and 5×10^{-5} M (○) mercuric chloride by D-penicillamine. Each *point* represents the mean of three different experiments ± standard error of the mean corrected for the corresponding D-penicillamine-treated control (●).

b. Relationship between D-penicillamine concentrations that cause 50% recovery of [^{3}H]QNB binding after inhibition with three levels of mercuric chloride. Data were obtained from a.

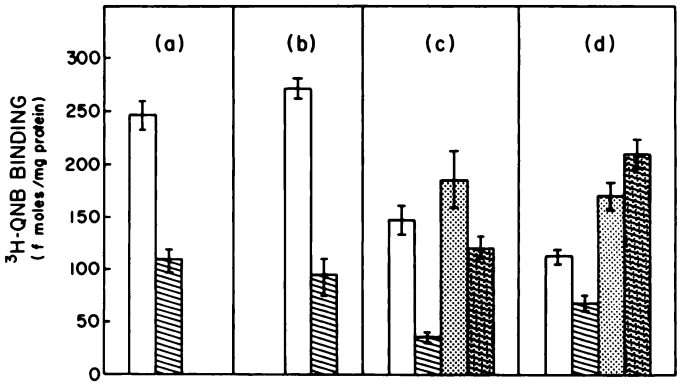

FIG. 5. *Regeneration of rat brain muscarinic receptor by* D-*penicillamine after inhibition with mercuric chloride*

Rat brain lysed synaptosomes (5 mg of protein) samples went through different treatments from *panels a* to *d*. Aliquots were taken from each sample in each treatment for the [^{3}H]QNB binding assay and protein determination. *Panel a* represents [^{3}H]QNB binding to rat brain synaptosomes treated with 5×10^{-5} M mercuric chloride for 1 hr (*striped bar*) compared with control (*open bar*). *Panel b* shows the same effect as in *a* after washing the free and loosely bound mercuric cations. *Panel c* shows the effect of D-penicillamine (10^{-3} M) treatment (for 1 hr) on [^{3}H]QNB binding of mercuric-untreated (*stippled bars*) and mercuric-treated (*striped + stippled bars*) washed synaptosomes in comparison with [^{3}H]QNB binding to control (*open bars*) and mercuric-treated (*striped bars*) washed synaptosomes. *panel d* represents the same procedure as in *c* except that all samples were washed to remove the free D-penicillamine and the soluble mercuric-D-penicillamine complexes from the incubation medium.

DISCUSSION

To characterize the molecular mechanism of inhibition of muscarinic acetylcholine receptor by methylmercury and mercuric chloride, it is important to utilize the available knowledge of the chemical and physical properties of the mercuric cation. Inorganic mercuric cation has a high polarizability value in aqueous solution as compared with cadmium and zinc (21). It also has high affinity for sulfhydryl groups, and at low pH the mercuric cation may bind exclusively protein—SH groups, whereas at high pH (>8.5), it may bind to other functional groups (22).

Results from the present work show that methylmercury and mercuric chloride block rat brain muscarinic acetylcholine receptors with different affinities. *In vitro*, mercuric chloride was found to be a significantly more potent inhibitor ($I_{50} = 1.6 \times 10^{-8}$ moles/mg of protein) than methylmercury ($I_{50} = 5.6 \times 10^{-6}$ moles/mg of protein). These differences in I_{50} values may reflect, in part, the physiocochemical properties of the organic and inorganic mercuric cations, where methylmercury is a monofunctional probe (binds to one—SH group) and mercuric chloride is a bifunctional probe (binds to two —SH groups).

The high affinity of mercuric cations to thiols provides a basis for the treatment of mercury poisoning with

antidotes, such as British anti-lewisite, which may form insoluble complexes with certain mercurials (21). This report demonstrates for the first time the inhibition and regeneration of a functional target in the brain cholinergic system by methylmercury and mercuric chloride. Regeneration of functionally active rat brain muscarinic receptors after inhibition with either methylmercury or mercuric chloride was achieved by D-penicillamine. The tightness of the bond between mercuric chloride and the receptor binding site was demonstrated by extensive washing before and after mercuric chloride treatment in relation to [^{3}H]QNB (Fig. 5a and b). [^{14}C]Methylmercury binds tightly to *Torpedo* nicotinic receptor and is not removed by dialysis for 48 hrs, but it can be displaced by British anti-lewisite or penicillamine (20). In the latter report, the direct correlation between the bound and displaced [^{14}C]methylmercury and the receptor functional activity (i.e., specific ligand binding) was not investigated. However, our results provide evidence that D-penicillamine chelates out mercuric cations that tightly bound to essential sulfhydryl groups in muscarinic receptor binding sites (as well as from non-essential —SH groups) and regenerates [^{3}H]QNB binding activity (Fig. 5c and d). The interaction between mercuric ions and D-penicillamine has been quantitatively measured.[2] It is possible that the muscarinic receptor binding site has two or more essential —SH groups. The mechanism by which the mercuric cation, as a bifunctional probe, blocks the [^{3}H]QNB binding site may include the formation of a —S—Hg—S—bridge involving two of the essential —SH groups in this site. The formation of this bridge may cause a change in the proper configuration of the binding site, preventing [^{3}H]QNB from binding, or it may mask the binding site. Methylmercury, as a monofunctional probe, binds to one —SH group only in the binding site, which may partially block (or retard) [^{3}H]QNB from binding to the receptor site. This may be the reason that more methylmercury than mercuric chloride is required to cause 50% inhibition of [^{3}H]QNB binding. In support to our results, the differential effects of NEM and *p*-chloromercuribenzoate on the agonist and antagonist binding to rat brain neural membrane also suggest the presence of —SH groups in the muscarinic receptor binding site (23). The purified nicotinic acetylcholine receptor from *Torpedo* has been shown to contain two —SH groups per binding site (8).

It is of interest that the delayed response of brain muscarinic receptors to low concentrations of either methylmercury or mercuric chloride may be due to the interaction of these thiol blockers with peripheral non-essential —SH groups in synaptosomal membrane vesicles. These sites may have to be saturated before interacting with the essential —SH groups in the muscarinic receptor binding sites. We previously (9) found that NEM enhances the inhibitory effects of methylmercury on brain muscarinic receptors. These results indicate that NEM treatment lowers the number of the peripheral free —SH groups which in turn lowers the concentration of methylmercury required for inhibition of [^{3}H]QNB binding.

We observed that D-penicillamine chelation of mercuric cations (organic and inorganic) leads to a complete retention of the proper configuration of the receptor binding site and [^{3}H]QNB binding (Fig. 5d). A higher D-penicillamine concentration is required to regenerate brain muscarinic receptors inhibited by mercuric chloride than that by methylmercury (Fig. 3). This observation also can be explained by the mono- and bifunctionality of methylmercury and mercuric chloride, respectively. The delay in regeneration of [^{3}H]QNB binding by D-penicillamine after inhibition with mercuric chloride (Figs. 3 and 4) may be due to chelation of mercuric cations from the peripheral non-essential —SH groups before those essential —SH groups in the binding site. Protection of rat brain acetylcholine muscarinic receptors against methylmercury and mercuric chloride also is achieved by D-penicillamine.[3]

Results from the present study and previous work (9) indicate that mercuric chloride is much more potent (100–350 times) inhibitor than is methylmercury on brain muscarinic receptors *in vitro*. These results provide evidence supporting the suggestion by Clarkson (24) that inorganic mercury could be the mediator of the neurotoxic effects of organomercurials. Earlier studies (25, 26) have shown that inorganic mercury detected in rat brain was about 4–7% of total methylmercury in the brain tissue after a single injection (i.p.) of methylmercury. Accordingly, if 1–5% of methylmercury is being biotransformed into inorganic mercury, it may cause 1–5 times more inhibition of muscarinic receptors than that caused by the total methylmercury concentration in the brain. It is important to emphasize that methylmercury can penetrate the blood-brain barrier more readily than can mercuric chloride. Demethylation of methylmercury may occur slowly in the brain, enhancing the inhibitory potential of methylmercury in the brain. This may be the reason that the neurological symptoms of methylmercury intoxication always appear after a latent period. Furthermore, similar pathological lesions in the central and peripheral nervous systems have been observed after injection with either mercuric chloride or methylmercury (27). It is also important to realize that air-exposed crystals or aged solutions of methylmercury may contain a small but potent amount of inorganic mercury.

Previous reports have shown that *N*-acetyl-DL-penicillamine (15) and D-penicillamine (16) and their mercury complexes are diffusible through the blood-brain barrier. Deacetylation of *N*-acetyl-DL-penicillamine leads to the release of the toxic DL-penicillamine, which may eliminate the clinical use of this drug in cases of mercury poisoning. On the other hand, D-penicillamine is nontoxic (11) and stable *in situ* (10) and, because of it solubility in water, it can cross the blood-brain barrier (16). Aaseth (15) reported that high concentrations of labeled mercury were found in the kidney after i.v. injection of CH_3-^{203}HgCl. Oral treatment with *N*-acetyl-DL-penicillamine for 7 days produced a decrease in the mercury levels of kidney, blood, liver, and brain. In the latter report, none of the animals treated for 7 days with *N*-acetyl-DL-penicillamine showed any signs of skin toxicity or weight loss. The clinical use of D-penicillamine as a drug in mercury intoxication is quite feasible for short-term treatment. If

[2] A.-S. A. Abd-Elfattah and A. E. Shamoo, unpublished data.

[3] A.-S. A. Abd-Elfattah and A. E. Shamoo, in preparation.

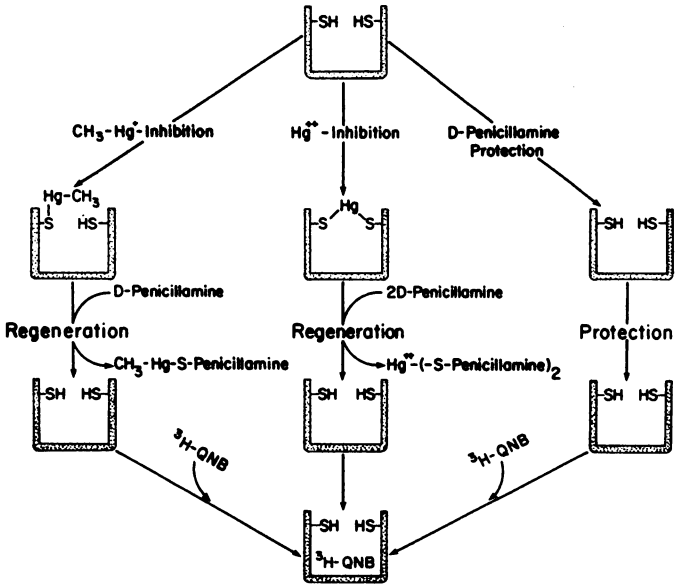

FIG. 6. *A hypothetical model of the interaction of methylmercury and mercuric chloride with muscarinic receptors*

The possible role of essential —SH groups in [^{3}H]QNB binding sites and their relative sensitivities to mercurials and regeneration of muscarinic receptors by D-penicillamine are shown in the model.

the kidney is in a functioning condition, the D-penicillamine treatment would be effective. This is explained by the fact that D-penicillamine treatment increases the urinary excretion of mercury (15). However, in cases of methylmercury or mercuric chloride intoxication where kidney damage exists, hemodialysis against cystein (28), gallbladder drainage, or oral treatment with nonabsorbable thiol resin (29, 30) would be more effective than D-penicillamine treatment. Our recent data[3] showed that D-penicillamine-methylmercury (1:1) and D-penicillamine-mercuric chloride (2:1) complexes are soluble in water and more likely to be excreted with the urine from a functional kidney.

A hypothetical model for the mechanism of interaction of methylmercury and mercuric chloride with "essential —SH groups" in the binding site of brain muscarinic receptor, and regeneration of a functionally active receptor by D-pencillamine, is illustrated in fig. 6. This model explains why the specific [^{3}H]QNB binding to muscarinic receptor which has been exposed to mercuric chloride and regenerated by D-penicillamine is consistently higher than the "controls" not exposed to mercuric chloride. The mercuric ion appears to stabilize or protect the binding site while the protein is subjected to experimental protocol. This may provide a basis for stabilization of the muscarinic receptors binding site during solubilization and purification of these receptors through subsequent treatment with mercuric chloride D-pencillamine.

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