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The Inhibition of Cerebral High Affinity Receptor Sites by Lead and Mercury Compounds

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Abstract. The effect of various concentrations of several lead and mercury compounds upon various high affinity receptor sites within discrete brain regions has been measured. The specific binding of radioactive spiroperidol and quinuclidinyl benzilate to striatal and cortical membranes respectively, was much more severely inhibited in the presence of tri-n-butyl lead acetate than by lead acetate. This suggested that the hydrophobic organic lead derivative was able to interfere with receptor structure more readily than the lead acetate. On the other hand mercuric chloride was more effective in blocking these two neurotransmitter receptor sites than was the organic methylmercuric chloride. This implied that sulfhydryl groups may be within, or proximal to the allosteric binding site. The relative ineffectiveness of all heavy metal compounds studied in blocking the glycine, GABA or the diazepam receptors indicated that the mechanism of binding may not be similar with different receptor proteins. Since micromolar concentrations of some lead and mercury compounds suffice to severely inhibit neurotransmitter binding sites, such a direct interference with postsynaptic events may in part account for the neurological consequences of heavy metal poisoning.

Key words: Lead; mercury – Receptors – Neurotransmitters – Heavy metals.

Introduction

The toxic effects of lead and mercury are especially pronounced at the neurological level (Felton et al. 1972; Rustam et al. 1975). The tremor, poor coordination and other movement disturbances caused by compounds containing these heavy metals, suggested that they may in part exert their effects by way of interference with nerve function. It has been previously reported that lead and mercury derivatives are able to modulate presynaptic phenomena such as the

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high affinity uptake and calcium-stimulated release of neurotransmitters (Silbergeld et al. 1979; Bondy et al. 1979a, b). In these latter studies, tri-n-butyl lead acetate was much more effective in disturbing such translocations than was lead acetate or inorganic and organic mercury containing compounds. Furthermore the dopamine system was more sensitive to these metals than were a series of other neurotransmitters. Other reports also suggest that organic lead poisoning presents a distinctive neuropathological picture which is not similar to organic tin compounds (Sturrock 1979).

In the present study we are reporting the effects of lead and mercury compounds upon the high affinity binding sites within brain tissue. These sites are specific toward various transmitter-related compounds and other pharmacological agents. Our data suggest that the chemical nature of each binding site varies considerably. The binding region of different receptors probably does not possess a common underlying peptide sequence.

Materials and Methods

Six week old male Sprague-Dawley rats were killed by CO_2 asphyxiation, decapitated, and their brains removed and placed on ice. Regions were dissected out using the protocol of Glowinski and Iversen (1966) as a guide, and then weighed and frozen at −40° C. Tissues were then homogenized in 19 volumes 0.32 M sucrose and centrifuged (40,000 *g*, 10 min). The precipitate was then resuspended in deionized water. A further centrifugation yielded the final residue which was either stored at −20° C or suspended in Tris buffer (40 mM pH 7.4) at a final concentration representing 50 mg original wet tissue/ml. Cerebellar membranes received an additional water wash prior to use.

Binding studies were carried out in a 1 ml incubation mixture containing 40 mM Tris pH 7.4 and a labeled pharmacological agent in the presence of various amounts of a heavy metal compound. In order to determine the extent of non-specific binding a series of incubations was performed in the presence of an excess of a non-radioactive competing ligand. The concentrations of the radioactive agents and the appropriate competing chemical used is given in Table 1. Isotopically labeled compounds were from New England Nuclear Corp., Boston, MA, USA. Specific activities (in Ci/mmol) were [1-phenyl-4-^{3}H]-spiroperidol (23); [methyl-^{3}H]-diazepam (73); [benzilic 4,4′-^{3}H(N)]-quinuclidinyl benzilate (29.4); [methylene-^{3}H(N)]-muscimol (7.3); [propyl 2,3-^{3}H]-dihydroalprenolol (46); [G-^{3}H]-strychnine (13); [9,10-^{3}H(N)]-dihydro-α-ergocryptine (21). Incubation was at 37° C for 10 min. The amount of tissue per incubation corresponded to 5 mg original wet tissue and contained around 400 μg protein. Protein concentration was determined by the method of Lowry et al. (1951). At the end of incubation, samples were filtered on glass fiber filters (25 mm diameter, 0.3 μm pore size Gelman Inc., Ann Arbor, USA) and washed three times rapidly with 5 ml of tris buffer at 0° C, except in the case of ^{3}H-strychnine which was only washed twice. Filters were then dried and counted in 5 ml of Aquasol (New England Nuclear Corp., Boston, MA; USA) scintillation mixture, at an efficiency of 38–43%, in a Packard Model 2660 liquid scintillation spectrometer.

Preliminary studies were carried out establishing the appropriateness of the above conditions. Such experiments included ascertaining that kinetic equilibrium was reached during the incubation time, that binding was reversible and proportional to the amount of membrane present and that the proportion of specific binding was between 60% and 94% of total binding. In addition, regional distribution studies of receptor density confirmed the selective nature of binding. The stereospecificity of the spiroperidol binding was demonstrated using D and L butaclamol as competing agents (Agrawal et al. 1980; Bondy 1980). The brain regions used for membrane preparation were chosen as containing a relatively high proportion of the receptor species being assayed (Table 1).

Each data point presented represents data obtained from three individual animals, each carried out in triplicate. Membranes for each point were prepared on three separate occasions. This

Table 1. Listing of receptor species assayed, pharmacological agents used and brain regions from which membranes were prepared

Receptor species	^{3}H-labeled ligand	Concentration (nM)	Unlabeled competitor	Concentration (μM)	Brain region
Dopamine	Spiroperidol	1.0	Haloperidol	1.0	Striatum
Glycine	Strychnine	1.0	Strychnine	10.0	Pons-medulla
Benzodiazepine	Diazepam	0.75	Diazepam	1.0	Cerebellum
Muscarinic, cholinergic	Quinuclidinyl, benzilate	1.0	Atropine	1.0	Cerebral cortex
GABA	Muscimol	1.0	GABA	1.0	Cerebellum
α-adrenergic	Dihydroergocryptine	1.0	Ergocryptine	1.0	Striatum

increased the variability of data but presumably more closely reflects the true alterations of binding than would selection of a representative sample.

Results

The heavy metal compounds studied differed considerably in their ability to influence ligand-receptor binding interactions when they were present in the incubation mixture. In addition, the individual receptors exhibited widely varying responses to the presence of these compounds. Binding of strychnine to the glycine receptor was not inhibited by any compound to an extent greater than 26% (Fig. 1). In this case, the more ionic compounds (lead acetate and mercuric chloride) appeared to have no effect on binding over the concentrations studied while the more polar compounds (tri-n-butyl lead acetate and methylmercuric chloride) had a slight inhibitory effect. Similarly, while the binding of diazepam to cerebellar membranes was somewhat inhibited by these compounds, this effect was under 40% inhibition and showed no clear dose-response relation (Fig. 2). This lack of dose-response kinetics suggested that the diazepam receptor might exist in two classes, one of which was very sensitive to heavy metal inhibition while the other was refractory to such interference.

The more hydrophilic heavy metal compounds had a greater effect on inhibition of ^{3}H-muscimol binding to the GABA receptor than the more organic tri-n-butyl lead acetate or methylmercuric chloride (Fig. 3). However, this receptor class was not inhibited by more than 35% under any of the conditions reported here.

Clearer dose-response relationships could be seen in the case of the striatal dopamine receptor (Fig. 4). While tri-n-butyl lead acetate inhibited ^{3}H-spiroperidol binding at concentrations down to 5×10^{-6} M, lead acetate was not inhibitory at 10^{-5} M. On the other hand the inhibitory effect of mercuric chloride and 5×10^{-6} M was over twice as great as that of methylmercuric chloride at the same concentration.

The inhibition of muscarinic receptors of cortical origin by heavy metal compounds was very similar to that observed for dopamine. Here again the more

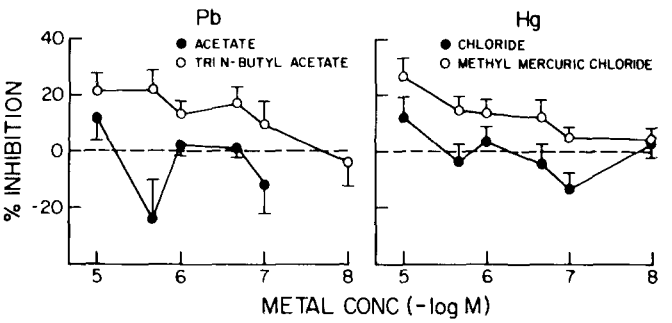

Fig. 1. Inhibition of ^{3}H-strychnine binding to pons-medulla membranes by various concentrations of heavy metal compounds. ● = Lead acetate or mercuric chloride. ○ = Tri-n-butyl lead acetate or methylmercuric chloride. Bars represent standard errors

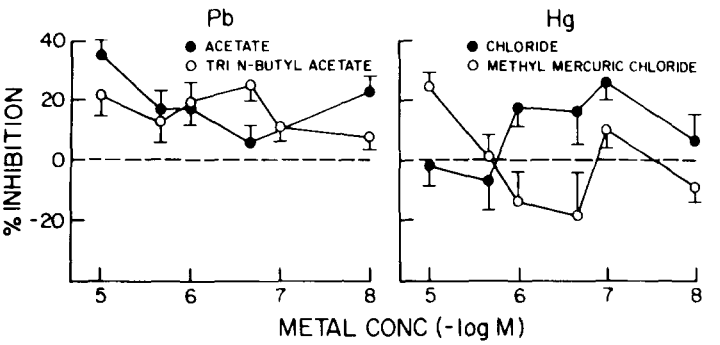

Fig. 2. Inhibition of ^{3}H-diazepam binding to cerebellar membranes by various concentrations of heavy metal compounds. ● = Lead acetate or mercuric chloride. ○ = Tri-n-butyl lead acetate or methylmercuric chloride. Bars represent standard errors

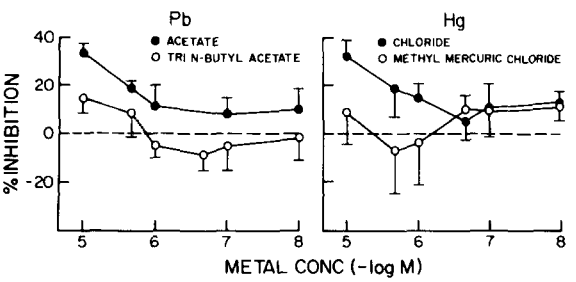

Fig. 3. Inhibition of GABA binding to cerebellar membranes by various concentrations of heavy metal compounds. ● = Lead acetate or mercuric chloride. ○ = Tri-n-butyl lead acetate or methylmercuric chloride. Bars represent standard errors

polar lead derivative was much more inhibitory than lead acetates. Conversely the more polar methylmercuric chloride had no inhibitory ability while mercuric chloride strongly blocked binding at 5×10^{-6} M (Fig. 5). Similar inhibition profiles were also found for the α-adrenergic receptor assayed with ^{3}H-dihydroergocryptine (Figure not shown).

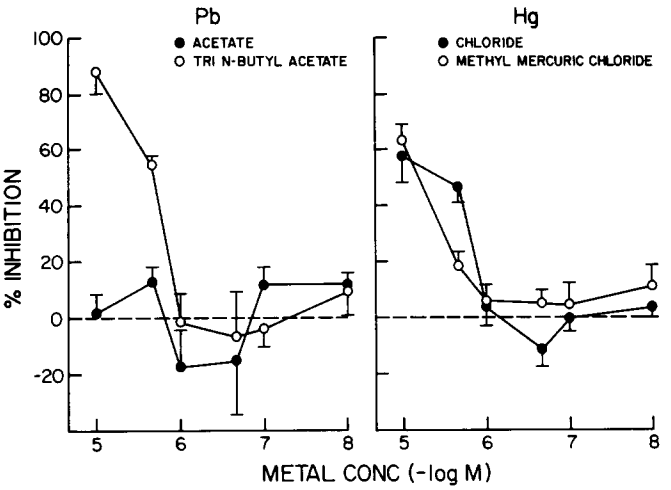

Fig. 4. Inhibition of ^{3}H-spiroperidol binding to striatal membranes by various concentrations of heavy metal compounds. ● = Lead acetate or mercuric chloride. ○ = Tri-n-butyl lead acetate or methylmercuric chloride. Bars represent standard errors

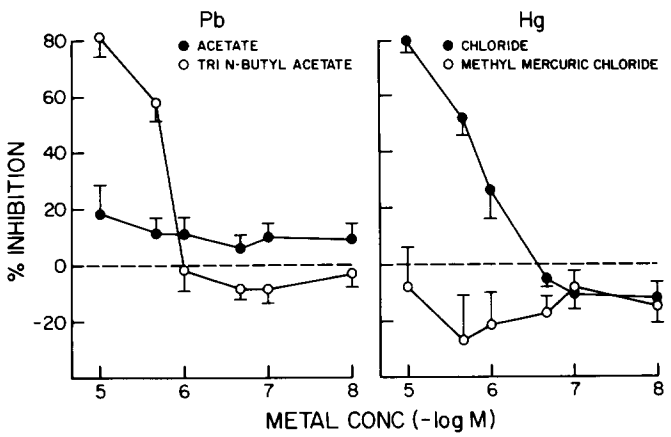

Fig. 5. Inhibition of ^{3}H-QNB binding to cortical membranes by various concentrations of heavy metal compounds. ● = Lead acetate or mercuric chloride. ○ = Tri-n-butyl lead acetate or methylmercuric chloride. Bars represent standard errors

Discussion

The responses of cerebral receptors to the presence of heavy metal derivatives are diversified. This is in contrast to the effects of these derivatives upon high affinity transport and calcium-stimulated release mechanisms. These latter phenomena are broadly similar for several different neurotransmitter translocations in their response to a given metal-containing compounds (Bondy et al. 1979a, b). This suggests that receptor species are molecularly dissimilar while

trans-membrane transport processes, although specific, may possess an underlying commonality.

The ability of tri-n-butyl acetate to be more inhibitory than lead acetate to the muscarinic and dopaminergic receptors may be due to its more nonpolar nature. This hydrophobic character may enable such a molecule to enter lipid membranes in order to interact with the binding region within the receptor molecule. Differences in receptor responses to more or less lipophilic molecules may reflect a greater or lesser concentration of non-polar amino acid residues within the location of the allosteric binding site.

The similar inhibition curves of dopamine and muscarinic acetylcholine receptor sites suggests that the binding sites of these receptor molecules may resemble each other. It should be borne in mind that the dopamine receptor within the striatum is known to be heterogeneous (Nagy et al. 1978; Schwarcz et al. 1978) while the muscarinic receptor also exists in pre- and postsynaptic regions (Szerb et al. 1977; Aguilar et al. 1979). The effect of various heavy metals upon the muscarinic has been previously reported (Aronstam and Eldefrawi 1979) and mercuric chloride was found to be much more effective in vitro than lead nitrate. These authors have also demonstrated the presence of sulfhydryl groups in rat brain muscarinic receptors and attribute the excess inhibition of mercury over lead to interaction at these sites (Aronstam et al. 1978). The critical nature of -SH groups to the muscarinic receptor binding site has also been shown by Hurko (1978).

The relevance of such in vitro studies to the whole animal situation is illustrated by two findings. The inhibition of muscarinic receptor binding induced by in vivo exposure to cadmium is similar to that seen by parallel in vitro studies (Hedlund et al. 1979). In addition, the concentrations of lead and mercury that are effective in inhibiting muscarinic receptors in vitro approximate those which occur in metal poisoning of the brain (Rustam et al. 1975; Aronstam and Eldefrawi 1979). However, in one study, lead-treated animals have been found to exhibit no changes in the level of the striatal dopamine receptor (Govoni et al. 1979). Furthermore heavy metals may owe their neurotoxic effects to indirect mechanisms. An example of this is the effect of lead upon the production of porphyrin by various organs, especially the erythropoetic tissue of the bone marrow. This stimulation may account for the resemblance of lead intoxication to porphyria (Silbergeld and Lamon 1980). In vitro studies cannot detect the substantial multi-organ interactions which frequently occur in systemic toxicity. However, the in vitro approach can be a useful adjunct where less parameters are studied under more controlled and better understood conditions than is the case with the intact animal.

The inhibition of dopamine-stimulated adenyl cyclase by the four metal compounds used in this study, closely parallels their inhibition of ^{3}H-spiroperidol binding. Thus lead acetate at 10^{-4} M has little effect on this enzyme while 5×10^{-6} M tri-n-butyl lead acetate inhibits adenyl cyclase by around 50%. Conversely the organic mercurial, methylmercuric chloride is less inhibitory to the cyclase than mercuric chloride (Wilson 1980), Thus the inhibition of dopamine-stimulated adenyl cyclase by heavy metals may be by way of interference with agonist-receptor interactions. The effects of organic and

inorganic lead compounds on the central nervous system resemble the effects of anticholinergic drugs in that they include ataxia, restlessness, irritability and confusion (Abood 1968; Kehoe 1976; Aldridge 1978). However, several of these signs may be associated with dopaminergic impairment (Silbergeld and Goldberg 1975; Reiter and Ash 1976; Govoni et al. 1979; Dubas et al. 1978). Exposure to lead is extremely prevalent (Grandjean 1978) and it may be that the inhibition of transmitter binding reported here accounts in part for some of the disturbances associated with heavy metal poisoning. On the other hand the dopamine receptor is sensitive to micromolar amounts of manganese (Usdin et al. 1980) which is known to adversely affect dopaminergic function (Goldman 1972; Schunk 1979).

References

Abood LG (1968) The psychomimetic glycolate esters. In: Burger A (ed) Drugs affecting the central nervous system. Dekker, New York, pp 127–167

Agrawal AK, Squibb RS, Bondy SC (1980) The effects of acrylamide upon the dopamine receptor. Toxicol Appl Pharmacol (in press)

Aguilar JS, Criado M, DeRobertis E (1979) Pre- and postsynaptic localization of central muscarinic receptors. Eur J Pharmacol 57: 227–230

Aldridge WN (1978) The biological properties of organogermanium, -tin and -lead compounds. In: Gielen M, Harrison PG (eds). The organometallic and coordination chemistry of germanium, tin and lead, pp 9–31

Aronstam RS, Abood LG, Hoss W (1978) Influence of sulfhydryl reagents and heavy metals on the functional state of the muscarinic acetylcholine receptor in rat brain. Mol Pharmacol 14: 575–586

Aronstam RS, Eldefrawi ME (1979) Transition and heavy metal inhibition of ligand binding to muscarinic acetylcholine receptors from rat brain. Toxicol Appl Pharmacol 48: 489–496

Bondy SC (1980) Rapid screening of neurotoxic agents in in vivo and in vitro means. Proc 5th Ann FDA Sci Symp (in press)

Bondy SC, Harrington ME, Anderson CL, Prasad KN (1979) The effect of low concentrations of an organic lead compound on the transport and release of putative neurotransmitters. Toxicol Letters 3: 35–41

Bondy SC, Anderson CL, Harrington ME, Prasad KN (1979b) The effects of organic and inorganic lead and mercury on neurotransmitter high affinity transport and release mechanisms. Environ Res 19: 102–111

Dubas TC, Stevenson A, Singhal RL, Hrida PD (1978) Regional alterations of brain biogenic amines in young rats following chronic lead exposure. Toxicol J 185–190

Felton JS (1972) Heavy metal poisoning: mercury and lead. Ann Intern Med 76: 779–792

Glowinski J, Iversen, LL (1966) Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-DOPA in various regions of the brain. J Neurochem 13: 655–669

Goldman M (1972) Levo-dihydroxyphenylalanine. Parkinson's disease and manganese poisoning. Indust Med 41: 12–17

Govoni S, Memo M, Spano PF, Trabucchi M (1979) Chronic lead treatment differentially affects dopamine synthesis in various rat brain areas. Toxicology 12: 343–349

Grandjean P (1978) Widening perspectives of lead toxicity. A review of health effects of lead exposure in adults. Environ Res 17: 303–321

Hurko O (1978) Specific ^{3}H-QNB binding activity in digitonin-solubilized preparations from bovine brain. Arch Biochem Biophys 190: 434–445

Kehoe RA (1976) Pharmacology and toxicology of heavy metals. Pharmacol Ther 1: 161–188

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Nagy JI, Lee T, Seeman P, Fibiger HC (1978) Direct evidence for presynaptic and postsynaptic dopamine receptors in the brain. Nature 274: 278–281

Reiter LW, Ash ME (1976) Neurotoxicity during lead exposure in the rat. Toxicol Appl Pharmacol 37: 160–165

Rustam H, VonBurg R, Amin-Zaki L, El Hassani S (1975) Evidence for a neuromuscular disorder in methylmercury poisoning. Arch Environ Health 30: 190–195

Schunk W (1979) Problems associated with chronic occupational manganese poisoning. Dtsch Gesundsheitswes 34: 1631–1633

Schwarcz R, Creese I, Coyle JT, Snyder SH (1978) Dopamine receptors localized on cerebral cortical afferents to rat corpus striatum. Nature 271: 766–768

Silbergeld EK (1977) Interactions of lead and calcium on the synaptosomal uptake of dopamine and choline. Life Sci 20: 309–318

Silbergeld EK, Goldberg AM (1975) Pharmacological and neurochemical investigations of lead-induced hyperactivity. Neuropharmacology 14: 431–444

Silbergeld EK, Lamon JM (1980) Role of altered heme synthesis in lead neurotoxicity. J Occupat Med (in press)

Sturrock RR (1979) A quantitative histological study of the effects of acute triethyl lead poisoning on the adult mouse brain. Neuropathol Appl Neurobiol 5: 419–431

Szerb JC, Hadhazy P, Dudar JD (1977) Release of [^{3}H] acetylcholine from rat hippocampal slices: Effect of septal lesion and of graded concentrations of muscarinic agonists and antagonists. Brain Res 128: 285–291

Usdin TB, Creese I, Snyder SH (1980) Regulation by cations of ^{3}H-spiroperidol binding associated with dopamine receptors of rat brain. J Neurochem 34: 669–676

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