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COMPETITIVE BINDING OF THE OXIMES HI-6 AND 2-PAM WITH REGIONAL BRAIN MUSCARINIC RECEPTORS

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Because of their ability to reactivate acetylcholinesterase in the peripheral nervous system, the oximes HI-6 and 2-PAM are used to treat organophosphorous poisoning. The central effects of these compounds may derive from their ability to interact with muscarinic cholinergic receptors.

Neural membranes were isolated from hippocampus, striatum and cortex, and the effects of HI-6 and 2-PAM on muscarinic receptor binding were assessed in a competitive binding assay using ^{3}H-QNB as the binding ligand. Both oximes were able to displace ^{3}H-QNB from the receptor with 2-PAM being about four times as potent as HI-6. The binding shows regional specificity and occurs at physiologically relevant concentrations, suggesting that these oximes directly effect central cholinergic receptors.

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PREFACE

The work described in this report was authorized under Project No. 1L162706A553(3A). This work was started in February 1983 and completed in October 1983. The experimental data are contained in laboratory notebooks 83-0027, 83-0105, 83-0135, and 83-0136 (Notebooks 1-4).

In conducting the work described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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COMPETITIVE BINDING OF THE OXIMES HI-6 AND 2-PAM WITH REGIONAL BRAIN MUSCARINIC RECEPTORS

1. INTRODUCTION

Most organophosphate (OP) poisoning is treatable with a combination of an antimuscarinic compound (e.g., atropine), and an oxime.[1] (((((4-aminocarbonyl)pyridino)methoxy)methyl)-2-((hydroxyimino)methyl)-pyridinium dichloride) (HI-6), one of a series of recently synthesized oximes designated H-oximes, reactivates soman-inhibited acetylcholinesterase (AChE) *in vitro*,[2] and soman-, but not tabun-inhibited neuromuscular function *in vivo*.

Investigators have suggested that the oximes penetrate the blood-brain barrier,[3,4] and indirect[5] and direct[6] evidence indicates that oximes are present in the brain after systemic injection. Rats poisoned with the alkyl-phosphate cholinesterase inhibitor O,O-dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate (Dipterex) show pyridine 2-aldoxime methiodide's (2-PAM's) increased entry into the central nervous system (CNS) relative to controls.[6] Structural barriers do not seem to be a factor, and the data suggest the existence of an active transport system.

Oximes, including 2-PAM and N,N'-oxydimethylene bis (pyridinium 4-aldoxime)dichloride, (toxogonin) are able to reactivate brain AChE following intracarotid injection, and a good correlation exists between the *in vivo* and *in vitro* results.[4] However, Clement[7] could not establish a correlation between the AChE reactivation of N-methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride (pro-PAM) relative to pyridine-2-aldoxime chloride (PAM), and survival of OP-poisoned animals. Therefore, while it is likely that the peripheral neuromuscular effects of oximes are due to their ability to reactivate AChE, other mechanisms may be involved in the CNS. Antimuscarinic properties of toxogonin and its structural analogs have been reported[8] in mice, suggesting that competitive interactions with cholinergic receptor mechanisms may be involved in the CNS effects of these compounds.

The present study tests this hypothesis in competitive, receptor binding assays using tritiated quinuclidinyl benzilate (^{3}H-QNB) and either 2-PAM or HI-6 as competitive ligands. Binding assays utilized membranes isolated from hippocampus (HIP), striatum (STR) and cortex (COR) of rat brain, areas enriched in synaptic cholinergic activity and known to be involved in symptoms of OP poisoning.

2. MATERIALS AND METHODS

2.1 Tissue Preparation.

Rats (AMRI (SD x WI)) BR, male; N=4) were decapitated and their brains were rapidly removed to an ice-cold dissecting plate. The HIP, STR and COR were dissected, weighed, and frozen in aluminum foil overnight (-70°C). Then, the tissues were thawed, and samples of approximately 60 mg were weighed and homogenized by polytron (Brinkman, setting 6, 10 sec) in 5 ml sodium-potassium phosphate-EDTA buffer (pH 7.4). The homogenates were incubated at 30°C for 15 minutes and placed on ice.

2.2 Receptor Binding Assay.

The following ingredients were combined in test tubes: 1.76 ml sodium potassium phosphate-EDTA buffer; 200 ul (about 2.5 mg) tissue homogenate of either HIP, STR, or COR from each of four rats, for a total of 12 tissue samples; 20 ul ^{3}H-QNB (0.21×10^{-9}M final concentration, 31 Ci/mM, Amersham); 20 ul of 2-PAM or HI-6 in one of 11 concentrations, plus an ethanol blank. The final concentrations of the oxime inhibitors were: 1×10^{-3}, 8×10^{-4}, 4×10^{-4}, 2×10^{-4}, 1×10^{-4}, 8×10^{-5}, 4×10^{-5}, 2×10^{-5}, 1×10^{-5}, 8×10^{-6}, and 4×10^{-6}M.

The test tubes were mixed by vortex and incubated for 30 min at 30°C. The reaction was terminated by placing the tubes in an ice bath for 2 min and then aspirating their contents onto Whatman GF/B filter paper using a Brandel tissue harvester. The filters were washed three times with 5 ml cold physiological saline (0.9 percent), placed in Hang-in vials (Packard), immersed in 5 ml Formula-947 scintillation cocktail (New England Nuclear), shaken and counted in a Packard 300-C scintillation spectrometer at 64 percent efficiency.

2.3 Data Analysis.

Initially, the data were expressed as moles of ^{3}H-QNB binding per milligram of tissue and then converted to percent bound at each concentration of oxime competitor. The IC_{50} for each oxime was determined with a standard dose-response, semi-log plot, the log of the concentration (abscissa) being plotted against the percent bound (ordinate).

3. RESULTS

The IC_{50} values were 7×10^{-5}M, 8×10^{-5}M, and 9.6×10^{-5}M for 2-PAM; 2.8×10^{-4}M, 3.5×10^{-4}M, and 3.7×10^{-4}M for HI-6 in the HIP (Figure 1), STR (Figure 2) and COR (Figure 3), respectively. Overall, 2-PAM was about four times more potent than HI-6 as inhibitor of ^{3}H-QNB binding, and both oximes showed ascending potency as a function of brain region, the order being HIP, STR, COR.

4. DISCUSSION

Because intoxication may trigger an active transport system which enhances the oximes' penetration of the CNS,[6] HI-6 and 2-PAM compete with ^{3}H-QNB in an *in vitro* system for the muscarinic cholinergic receptor at concentrations comparable to levels which are likely to be achieved *in vivo* with a therapeutic dose administered in the event of OP poisoning. Therefore, in addition to any cholinesterase reactivating properties which these compounds possess, they also exert competitive effects at the muscarinic receptor. There are, however, problems associated with postulating the receptor activity of the oximes as the primary mechanism for their therapeutic efficacy. First, the lack of a relationship between antimuscarinic potency and therapeutic effect of atropine-like drugs would seem to argue against this interpretation; second, although HI-6 is more efficacious therapeutically than 2-PAM against Soman poisoning, 2-PAM is four times more potent than HI-6 in inhibiting ^{3}H-QNB binding.

The first problem may be a matter of the kinetics of interaction at the receptor. For example, the oximes and atropine may interact differentially

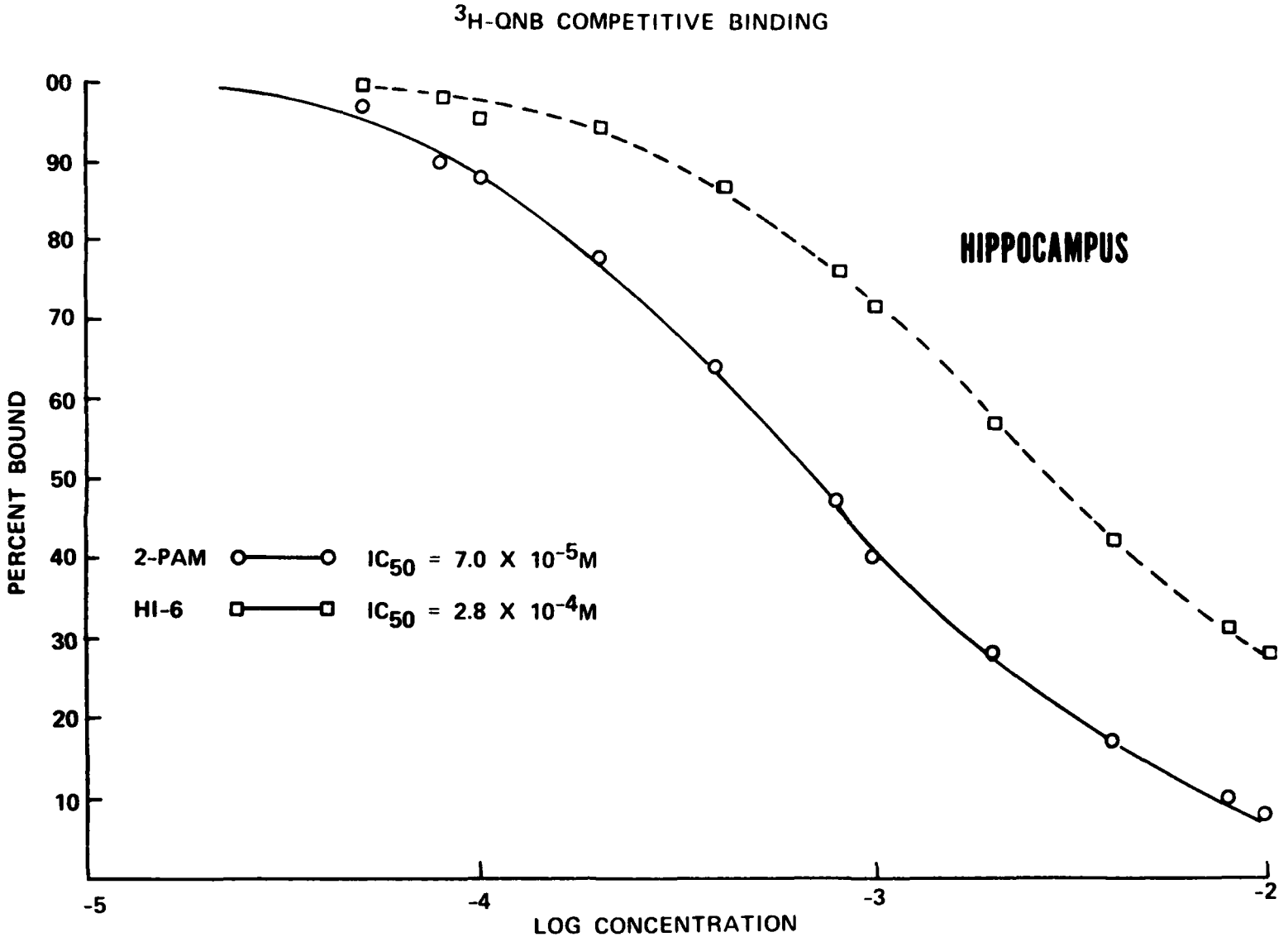

Figure 1. Competitive Binding of ^{3}H-QNB with Either HI-6 or 2-PAM in Neural Membranes Isolated from Hippocampus of Rats. Four Replications Per Data Point.

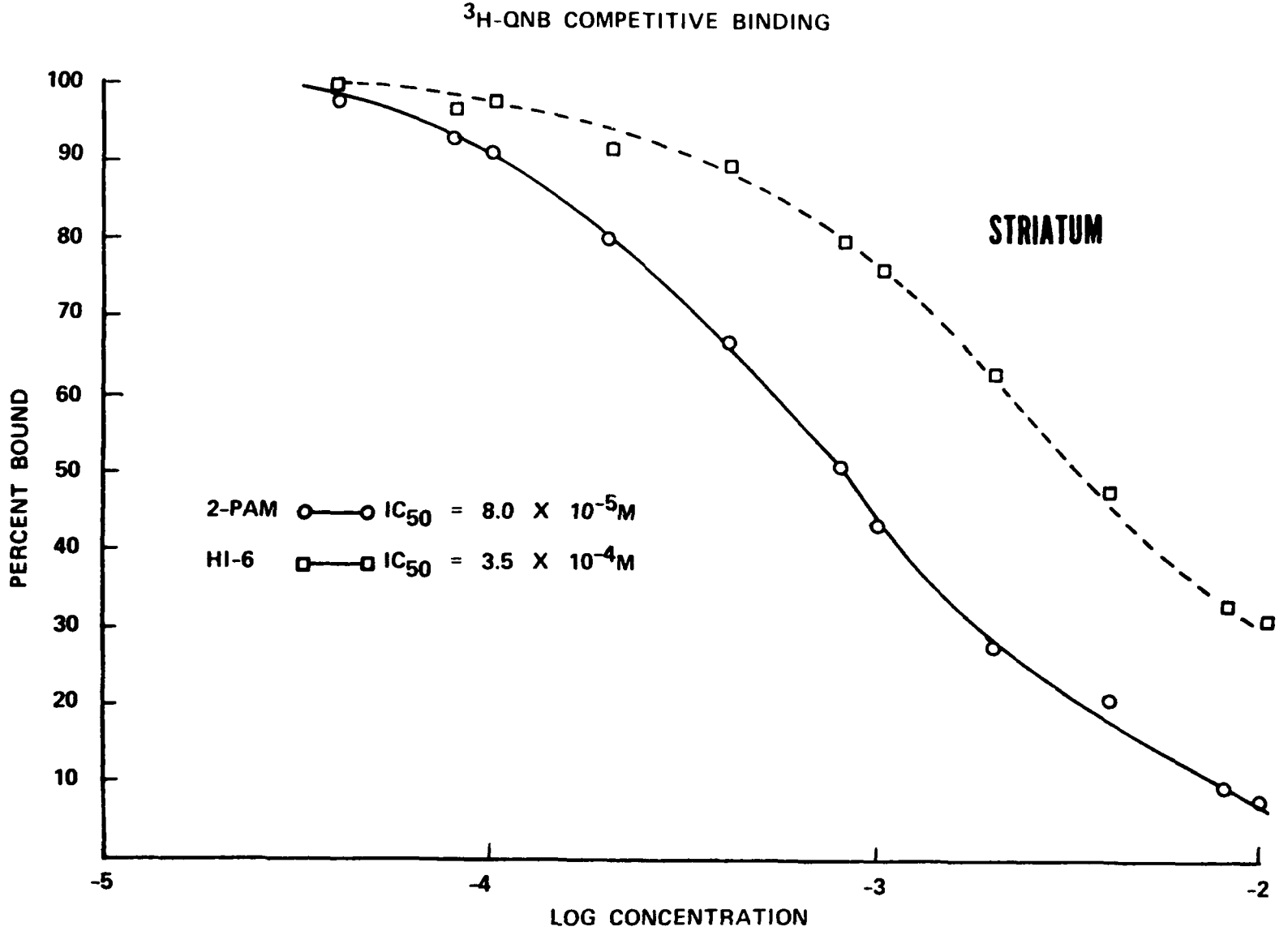

Figure 2. Competitive Binding of ^{3}H-QNB with Either HI-6 or 2-PAM in Neural Membranes Isolated from Striatum of Rats. Four Replications Per Data Point.

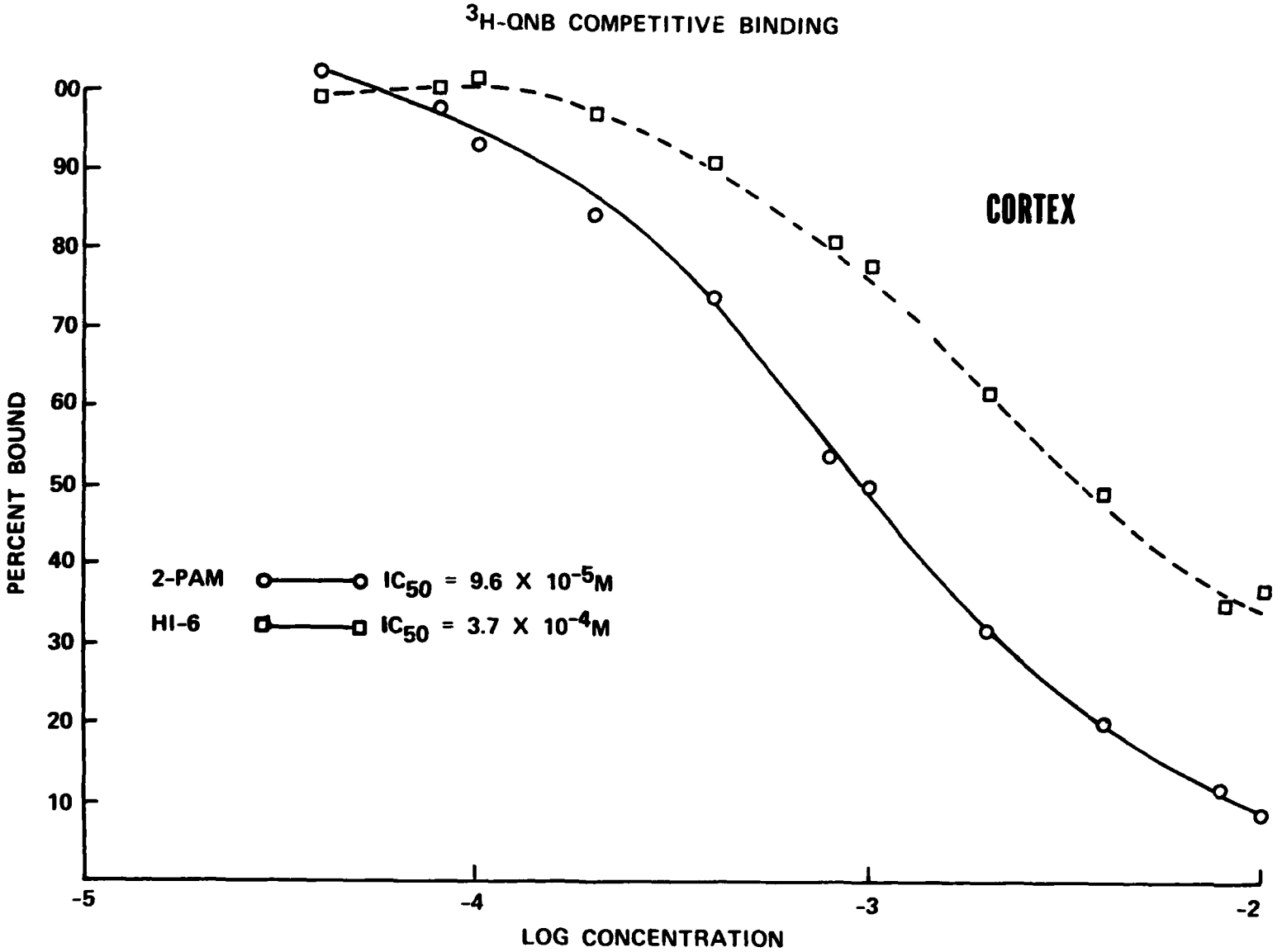

Figure 3. Competitive Binding of ^{3}H-QNB with Either HI-6 or 2-PAM in Neural Membranes Isolated from Cortex of Rats. Four Replications Per Data Point.

with the different subunits of the receptor, or the oxime effects may be due to allosteric hinderance rather than direct competition with ^{3}H-QNB for the same site. On the other hand, the oximes may exert qualitatively different physiological effects than atropine, acting as partial or pure agonists at the receptor. This latter possibility could be investigated with ion flux studies designed to test the effects on the ion channel of receptor occupation. The second problem, that of the greater binding of the therapeutically inferior 2-PAM, can be explained in one of two ways. First, 2-PAM's and HI-6's penetration of the CNS of intact organisms may be biased in favor of HI-6. This is possible, though unlikely, since brain levels of 2-PAM reach respectable (28-43 percent of that injected) levels in brains of rats challenged with the cholinesterase inhibitor Dipterex.[6] At present, no direct evidence on HI-6 penetrating the CNS has been reported. A more likely explanation is that the two oximes may have different agonist/antagonist properties at the receptor.

One other interesting observation was that the regional specificity of these oximes had an identical pattern, indicating that their ability to interact with the receptors was partially dependent upon the characteristics of the tissue. In approximate ascending phylogenetic order, the three brain regions representing different tissue types and stages of phylogenetic development are basal ganglionic (STR), allocortical (HIP), and cortical (COR). The functional and structural characteristics of GABA receptors have been shown to vary amongst these regions, and the same may be true of the cholinergic system.

5. CONCLUSIONS

The oximes, HI-6 and 2-PAM, can directly interact with the muscarinic cholinergic receptor as assessed by competitive in vitro receptor binding assay. This binding shows regional specificity of the oximes and may occur at physiologically relevant concentrations. Finally, the lack of relationship between therapeutic efficacy and receptor binding is probably due to differing agonist/antagonist properties of the oximes at the receptor.

LITERATURE CITED

1. Fleisher, J. H., Harris, L. W., Miller, G. R., Thomas, N. C., and Clift, W. J. Antagonism of Sarin Poisoning in Rats and Guinea Pigs by Atropine, Oximes and Mecamylamine. Toxicol. Appl. Pharmac. 16, 40 (1970).

2. De Jong, L. P. A., and Wolring, G. Z. Reactivation of Acetylcholinesterase Inhibited by Soman with HI-6 and Related Oximes. Biochem. Pharmac. 29, 2379 (1980).

3. Wolthuis, O. L., and Kepner, L. A. Successful Oxime Therapy One Hour After Soman Intoxication in the Rat. Eur. J. Pharmac. 49, 415 (1978).

4. De la Manche, J. S., Verge, D. E., Bouchard C., Coq, H., and Sentenac-Roumanou, H. Penetration of Oximes Across the Blood-Brain Barrier. A Histochemical Study of the Cerebral Cholinesterases Reactivation. Experientia. 35, 531 (1979).

5. Lundy, P. M., and Shih, T. M. Examination of the Role of Central Cholinergic Mechanisms in the Therapeutic Effects of HI-6 in Organophosphate Poisoning. J. Neurochem. 40, 1321 (1983).

6. Firemark, H., Barlow, C. F., and Roth, L. J. The Penetration of 14 C-2-PAM into Brain and the Effect of Cholinesterase Inhibitors on Its Transport. J. Pharmac. Exper. Thera. 145, 252 (1964).

7. Clement, J. G. Efficacy of Pro-PAM (N-Methyl-1,6-Dihydropyridine-2-Carbaldoxime Hydrochloride) as a Prophylaxis Against Organophosphate Poisoning. Toxicol. Appl. Pharmac. 47, 305 (1979).

8. Amitai, G., Kloog, Y., Balderman, D., and Sokolovsky, M. The Interaction of Bis-Pyridinium Oximes with Mouse Brain Muscarinic Receptor. Biochem. Pharmac. 29, 483 (1980).