isoproterenol > (+)-isoproterenol. For the related adrenergic amine, norepinephrine, both absence [18, 19] and presence [20] of stereospecificity has been reported.

Isoproterenol was the most potent inhibitor among the agonists with (−)-norepinephrine more potent than (−)-epinephrine, in accordance with an earlier study [20]. However, norepinephrine has also been reported to be less [17] or equally [18] bound in human serum as epinephrine. The differences in experimental procedures may explain the different results. Our observations indicate that the adrenergic agonists have affinity for the binding site on *alpha*-1 acid glycoprotein. Phenylephrine, classified as an *alpha*-adrenergic agonist, had no ability to inhibit radiolabelled propranolol binding.

The saturable binding site for propranolol in serum, probably mainly located on *alpha*-1 acid glycoprotein, has similarities to the *beta*-adrenergic receptor binding site: The antagonists are bound with considerably higher affinity than the agonists, both agonists and antagonists exhibit stereospecific binding, and the *beta*-adrenergic ligands were more potent than the *alpha*-adrenergic substances. However, great differences in binding affinity and specificity between the saturable binding site in serum and the *beta*-adrenergic receptor rule out the possibility of a solubilized and intact circulating receptor.

In the present study we have decomposed serum binding of radiolabelled propranolol into saturable and nonsaturable binding. The nonsaturable binding was not affected by the addition of other adrenergic ligands, in contrast to saturable binding that was inhibited both by antagonists and agonists. The order of inhibitory potency was: (±)-Hydroxybenzylpindolol > (−)-propranolol > (−)-alprenolol > prazosin = chlorpromazine for the antagonists and (−)-Isoproterenol > (−)-norepinephrine > (−)-epinephrine > (−)-phenylephrine for the agonists. The antagonists had markedly higher affinity for the binding site compared to the agonists, But stereospecificity was found for both antagonists and agonists with the levoform markedly more potent than the dextroform. Studies are in progress to characterize the common adrenergic binding site in human serum.

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Competitive binding of the oximes HI-6 and 2-PAM with regional brain muscarinic receptors

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Most organophosphate (OP) poisoning has been treatable with a combination of an anti-muscarinic compound, such as atropine, and an oxime, such as [({[(4-aminocarbonyl)-pyridino]methoxy)methyl]-2-[(hydroxyimino)methyl] pyridinium dichloride (HI-6) [1, 2]. The oximes probably penetrate the blood–brain barrier [3, 4], as both indirect [5]

and direct [6] evidence indicates the presence of oximes in the brain after systemic injection. Thus, 2-PAM (pyridine-2-aldoxime methiodide) and toxogonin [*N,N'*-oxydimethylene bis(pyridinium 4-aldoxime)dichloride] are able to reactivate brain AChE following intracarotid injection, and there appears to be a good correlation between the *in vivo* and *in vitro* results [4]. Anti-muscarinic properties of the bispyridinium oxime toxogonin and its structural analogs have been reported in mice [7], and the H-oximes (HGG series) block peripheral muscarinic receptors in guinea pig ileum at micromolar concentrations [8]. This suggests 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 crude homogenates of tissue 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.

Materials and methods

Rats (AMRI(SD × WI)BR, Male; N = 4) were decapitated and the brains were rapidly removed to an ice-cold dissecting plate. Homogenates were prepared by a modification of established procedures [9]. The HIP, STR and COR were dissected and weighed, and then frozen in aluminum foil overnight (−70°). The tissues were then thawed, and samples of approximately 60 mg were weighed and homogenized by polytron (Brinkmann, setting 6, 10 sec) in 5 ml sodium potassium phosphate-EDTA buffer (pH 7.4). The crude homogenates were then incubated at 30° for 15 min and placed on ice.

The following were combined in test tubes: 1.76 ml sodium potassium phosphate–EDTA buffer; 200 μl (about 2.5 mg tissue) tissue homogenate of HIP (158 μg protein), STR (186 μg protein), or COR (218 μg protein) from each of four rats, for a total of twelve tissue samples; 20 μl [^{3}H]-QNB (0.21×10^{-9} M final concn., 31 Ci/mmole, Amersham); and 20 μl of either 2-PAM or HI-6 in one of eleven 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 contents of the test tubes were then mixed by vortex and incubated for 30 min at 30°. 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 then washed three times with 5 ml of cold physiological saline (0.9%), placed in Hang-in vials (Packard), immersed in 5 ml Formula-947 scintillation fluid (New England Nuclear), shaken, and counted in a Packard 300-C scintillation spectrometer at 64% efficiency. The data were initially expressed as nanomoles of [^{3}H]QNB binding per mg 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).

Results and discussion

The IC_{50} values for 2-PAM were 7.0×10^{-5} M, 8.0×10^{-5} M, and 9.6×10^{-5} M, and for HI-6 were 2.8×10^{-4} M, 3.5×10^{-4} M, and 3.7×10^{-4} M in the HIP (Fig. 1), STR (Fig. 2) and COR (Fig. 3) respectively. Overall, 2-PAM was about four times more potent as an inhibitor of [^{3}H]QNB binding than was HI-6, and both oximes showed ascending potency as a function of brain region, the order being COR, STR, HIP.

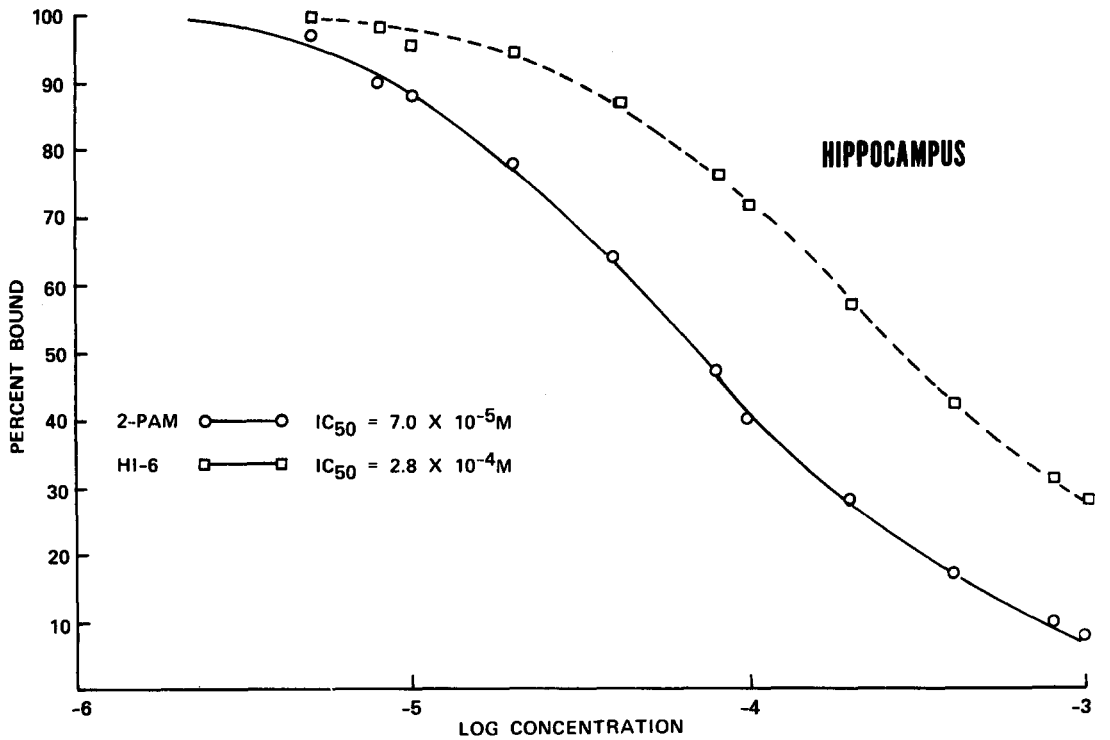

Fig. 1. Competitive binding of [^{3}H]QNB with either HI-6 or 2-PAM in neural membranes isolated from hippocampus of rats. Four replications per data point.

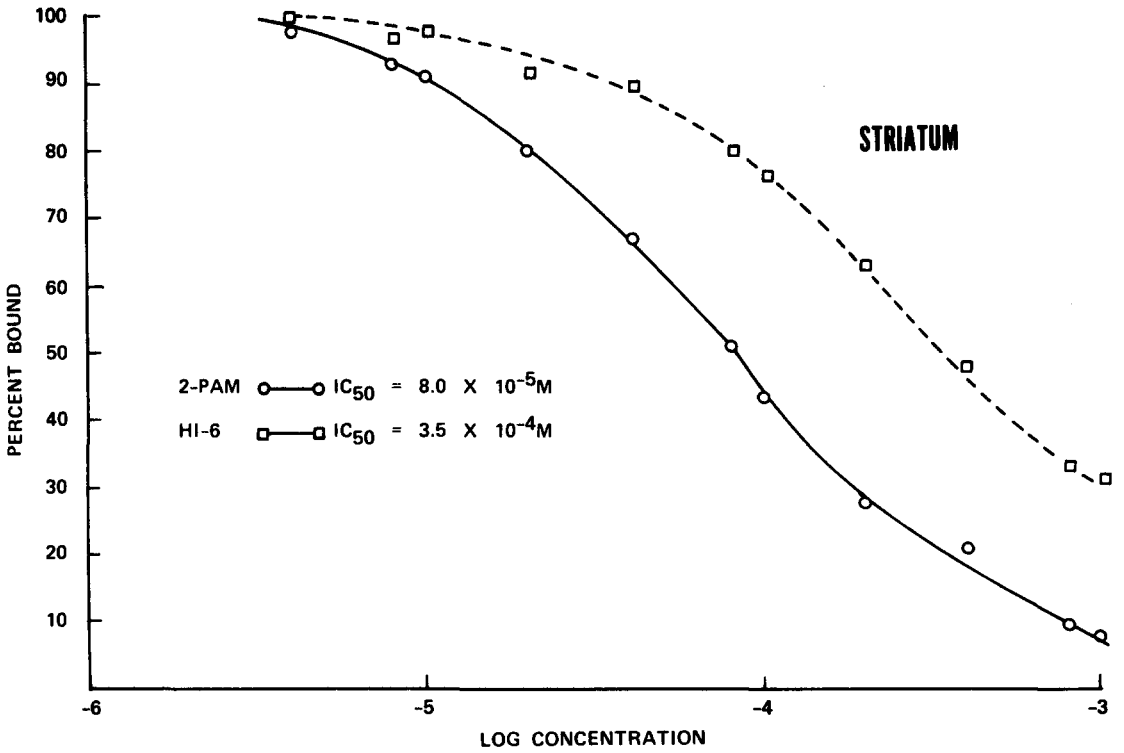

Fig. 2. Competitive binding of [^{3}H]QNB with either HI-6 or 2-PAM in neural membranes isolated from striatum of rats. Four replications per data point.

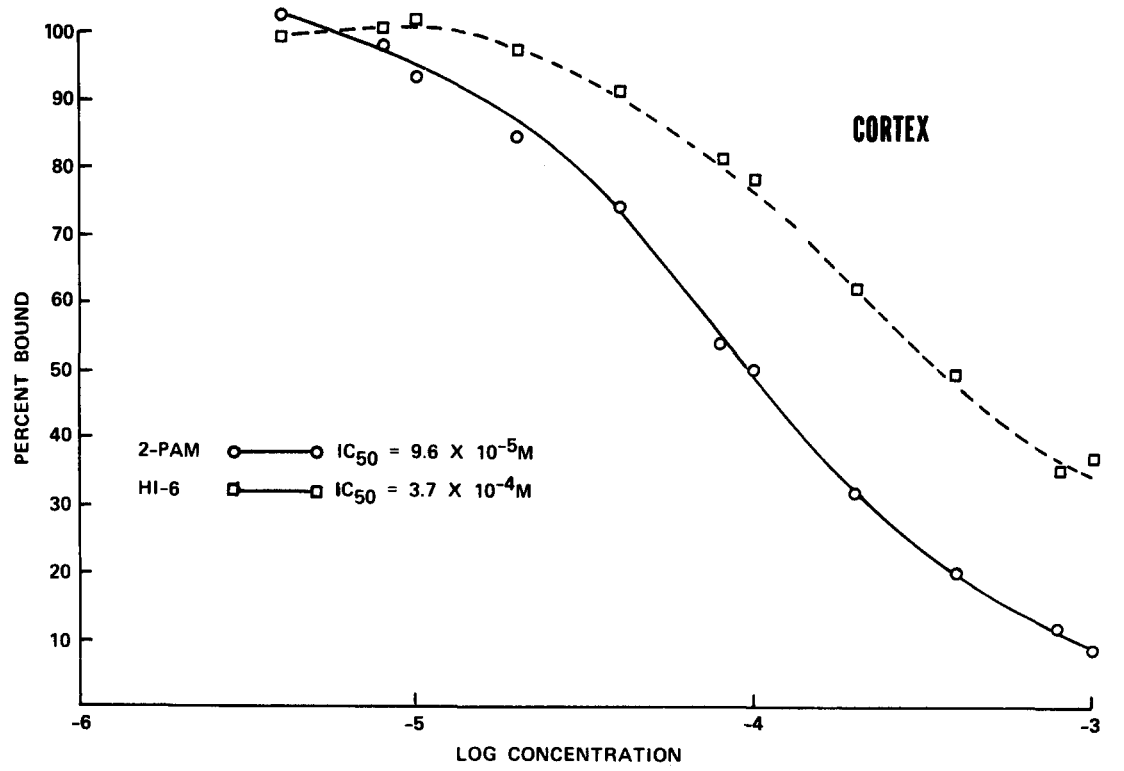

Fig. 3. Competitive binding of [^{3}H]QNB with either HI-6 or 2-PAM in neural membranes isolated from cortex of rats. Four replications per data point.

The oximes 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. For example, an injection of [^{14}C]2-PAM (20 mg/kg, i.v.) in rat resulted in plasma concentrations of the unmetabolized oxime of 49, 8.9 and 8.6 μM at 10 min, 1 hr, and 6 hr respectively. Calculations of unmetabolized oxime in brain regions were 5–12, 8–26 and 28–43% of plasma levels at these three time points [6]. Regions of greater vasculature (e.g. cortex) showed the highest levels, and these concentrations may be enhanced in the event of therapeutic intervention following OP poisoning. This is a reasonable inference because intoxication may trigger an active transport system which enhances penetration of oximes into the CNS [6]. 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 anti-muscarinic potency and therapeutic effect of atropine-like drugs would seem to argue against this interpretation; second, HI-6 is more therapeutically efficacious than 2-PAM against soman (pinacolyl methylphosphonofluoridate) poisoning, yet the latter shows four times more potency 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 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. The second problem, that of the greater binding of the therapeutically inferior 2-PAM, can be explained in one of two ways. First, penetration of 2-PAM and HI-6 into the CNS of intact organisms exposed to OPs may be biased in favor of HI-6 as a result of differential activation of a hypothetical transport system [6]. This is possible, though unlikely, since brain levels of 2-PAM reach respectable (28–43% of that injected) levels in brains of rats challenged with the cholinesterase inhibitor Dipterex [6]. The second, and more likely, explanation is, again, 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. These three brain regions represent different tissue types and stages of phylogenetic development, these being basal ganglionic (STR), allocortical (HIP), and cortical (COR), in approximate ascending phylogenetic order. The functional and pharmacological characterstics of γ-aminobutyric acid (GABA) receptors have been shown to exhibit regional variation [10], and the same may be true of the cholinergic system.

In summary, 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.

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