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Tetrahydro-9-aminoacridine (THA) interacts with the phencyclidine (PCP) receptor site

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The effect of tetrahydro-9-aminoacridine (THA) and related compounds on ligand binding to the dissociative anesthetic (phencyclidine, PCP) receptor site was assessed using a rat brain homogenate assay. THA displaced the dissociative anesthetic ligand [^{3}H]*N*-(1-[2-thienyl]cyclohexyl)3-4-piperidine ([^{3}H]TCP) binding with an IC_{50} of 26 μM. Other acridine derivatives displayed similar potency as displacers of [^{3}H]TCP. Cholinesterase inhibitors and aminopyridines had IC_{50}s equal to or greater than 100 μM. Saturation studies of [^{3}H]TCP in the presence and absence of 30 μM THA revealed competitive inhibition with a K_i of 15 μM. The clinical pharmacology of THA suggests that it antagonizes the effects of dissociative anesthetics whereas in vitro, it behaves as a weak PCP agonist. THA may exert some of its clinical effects through interaction with the PCP receptor and may have mixed agonist-antagonist properties.

Tetrahydro-9-aminoacridine (THA) is an orally administered, centrally active cholinesterase inhibitor recently reported to ameliorate significantly the cognitive deficits of dementia of the Alzheimer's type (DAT) [16]. While the effects of THA have been attributed to its action as a cholinesterase inhibitor, clinical trials of other cholinesterase inhibitors have yielded unimpressive results [6]. This discrepancy suggests that THA may have additional modes of action.

THA has a complicated pharmacology. It has been found to reverse morphine and magnesium chloride induced coma [13] and to attenuate the intensity of narcotic withdrawal symptoms [3, 15]. It is a potassium and sodium channel blocker [12, 14]. It has also been found to decrease the duration of anesthesia induced by the dissociative anesthetics, phencyclidine (PCP) and ketamine [1, 2, 4], as well as to reduce the emergence symptoms associated with ketamine anesthesia in both man and animals [1, 4].

PCP receptor density decreases have been documented in DAT [10, 11]. The PCP receptor is functionally associated with the *N*-methyl-D-aspartate (NMDA) subtype

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of excitatory amino acid receptors and NMDA receptor density also declines in DAT [8] NMDA receptors are particularly prominent in the hippocampus where the brunt of DAT pathology falls

Because THA apparently antagonizes some of the effects of PCP-like drugs, we studied the possible interaction of THA and structurally related compounds with the PCP receptor site We also studied the effects of other cholinesterase inhibitors and structurally related potassium channel blockers

Rat forebrain membranes were homogenized in 50 mM Tris-acetate buffer (pH 7 0 at 4°C) with a Polytron at a setting of 7 Membranes were then centrifuged for 20 min at 20,000 *g* The pellet was resuspended in 50 mM Tris-acetate buffer, (pH 7 0 at room temperature) Varying concentrations of THA and related compounds were incubated with prepared membranes and the specific PCP ligand [^{3}H]*N*-(1-[2-thienyl]-cyclohexyl)-3,4-piperidine (TCP) at a concentration of 10 nM Triplicate samples were incubated for 30 min at room temperature, then filtered over GF/B glass filters Filters were washed twice with 4 ml 50 mM Tris-acetate buffer (pH 7 0 at 4°C) All filters were pre-soaked in 0 5% polyethyleneimine to eliminate binding of [^{3}H]TCP to the filters Filters were immersed in 5 ml aqueous counting solution (Amersham) and radioactivity determined in a Beckman LS 8100 scintillation counter Non-specific binding was assessed with the addition of 10 μM TCP

THA displaced [^{3}H]TCP with an IC_{50} of 26 μM (Table I) The acridine derivatives 9-aminoacridine and proflavin were of comparable potency (Table I) The cholinesterase inhibitor neostigmine had an IC_{50} greater than 100 μM Two other cholinesterase inhibitors, physostigmine and edrophonium, had IC_{50}s of 100 μM Three amino

TABLE I

IC_{50} VALUES FOR THA AND 2 RELATED ACRIDINES, 3 CHOLINESTERASE INHIBITORS AND 3 AMINOPYRIDINES

The latter are potent potassium channel blockers THA and the related acridines inhibit [^{3}H]TCP binding with IC_{50}s in the low micromolar range Cholinesterase inhibitors and aminopyridines are considerably less potent (mean of 3 experiments)

Drug	IC_{50} (μM)
Acridines	
THA	26
9-Aminoacridine	9
Proflavin	20
Acetylcholinesterase inhibitors	
Physostigmine	100
Neostigmine	>100
Edrophonium	100
Aminopyridines	
4-Aminopyridine	>100
3,4-Diaminopyridine	>100
3-Aminopyridine	>100

pyridines, potent potassium channel blockers structurally related to THA, had IC_{50}s greater than 100 μM

To investigate the nature of the interaction of THA with the PCP receptor site we performed saturation curves for [^{3}H]TCP in the presence and absence of 30 μM THA For [^{3}H]TCP binding in the absence of THA, Scatchard analysis indicated a single class of binding sites with a mean K_d of 45 nM (S D = 7 64, n = 3) and mean B_{max} of 773 fmol/mg protein (S D = 194, n = 3) (Fig 1) In the presence of 30 μM THA the mean K_d was 117 nM (S D = 57 13, n = 3) and mean B_{max} was 748 fmol/mg protein (S D = 195, n = 3) (Fig 1), indicative of competitive inhibition Double-reciprocal plots confirmed competitive inhibition with a mean K_i of 15 μM (S D = 6 62, n = 3) At a concentration of 300 μM, THA had no effect on NMDA receptor binding in a sensitive autoradiographic assay (data not shown) Preliminary experiments indicate that THA also displaces the PCP ligand MK-801

Davenport et al have examined the effects of THA and related compounds on NMDA mediated neurotoxicity and neuroexcitation [7] They found that THA was a moderately effective antagonist of NMDA, that cholinesterase inhibitors were weak antagonists, and that the potassium channel blocker 4-aminopyridine had no NMDA antagonist properties This rank order of potency accords well with our binding data

Drugs active at the PCP receptor are thought to block NMDA responses noncompetitively through actions at the cation channel associated with the NMDA receptor [5] THA is a known potassium and sodium channel blocker and the closely related compound 9-aminoacridine is a sodium channel blocker [17] THA reduces the

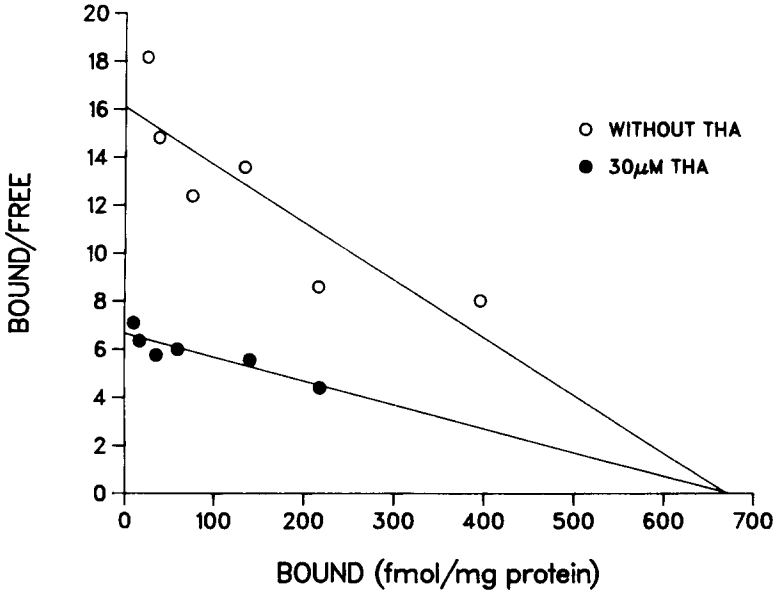

Fig 1 Scatchard plot demonstrating competitive inhibition (representative plot from one of 3 experiments)

duration of anesthesia and emergence symptoms associated with dissociative anesthesics This suggests that THA antagonizes the effects of dissociative anesthetics These data, together with those of Davenport et al , suggest that THA may have mixed agonist–antagonist properties at the PCP receptor No PCP antagonist has yet been found and such an agent could be of great interest

The antagonism of THA for NMDA could limit the effectiveness of THA in DAT Decreases in PCP/NMDA receptors in DAT are particularly prominent in the hippocampus In addition to being a focus of pathology in DAT, it is known that the major afferent projection to the hippocampus, the perforant pathway, is seriously affected in DAT [9] This pathway is felt to be glutamatergic and deficiencies in glutamate neurotransmission may contribute to the memory deficits of DAT Further decreases in NMDA mediated neurotransmission in the hippocampus could exacerbate the memory deficits of DAT On the other hand, should THA prove to have PCP antagonist properties it could potentiate NMDA receptor neurotransmission Even a modest increase in the efficiency of glutamatergic neurotransmission in the hippocampus could be of benefit in DAT

Finally, it should be pointed out that the K_i of THA for TCP binding is considerably higher than the reported effective serum concentration of THA The effective brain concentration is unknown and a proper assessment of the relevance of our findings awaits determination of this value

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