Alzheimer's disease (AD), a slowly progressive neuro-psychiatric illness characterized by memory deficits, is the major cause of dementia among the elderly (Katzman, 1986). The disease is associated with a loss of central cholinergic cells (Davies & Maloney, 1976), and in some cases a nor-adrenergic deficit (Bondareff et al., 1982). Recently, Summers et al. (1986) reported that THA (1,2,3,4-tetrahydro-9-aminoacridine; Tacrine), a potent, centrally acting cholinesterase inhibitor, improved symptoms in patients with moderate to severe AD; however, liver toxicity appears to be a limiting factor. The 1-hydroxyl derivative of THA (HP 029) was synthesized in our laboratories (Shutske et al., 1988) in an attempt to reduce the toxicity of THA. By providing a hydroxyl group for glucuronide conjugation, elimination might be facilitated and toxicity limited, hopefully without sacrificing cholinesterase inhibitory activity. In this regard, HP 029 was found to be active in pharmacological tests yet far less toxic than THA in acute and subchronic animal toxicology (Shutske et al., 1988). The unique high-affinity mechanism of reversible, non-covalent inhibition by which THA inhibits cholinesterase (Steinberg et al., 1975) further increased our interest in THA-like compounds. In this report, we present the results of biochemical studies with a series of HP 029 analogues.

Acetylcholinesterase (AChE) was measured in rat striatal homogenates by a modification of the method described by Ellman et al. (1961) using acetylthiocholine as substrate. Butyrylcholinesterase (BChE) was determined in a similar manner, except that butyrylthiocholine was used as the substrate and human serum (Prestipic, Biodynamics, Houston, TX) as a source of the enzyme. Biogenic amine reuptake was measured in rat brain synaptosomes by a modification (Meyerson et al., 1980) of the procedure described by Snyder & Coyle (1969).

Abbreviations used: AD, Alzheimer's disease; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; NA, noradrenaline; THA, 1,2,3,4-tetrahydro-9-aminoacridine.

9-Amino-1,2,3,4-tetrahydroacridine-1-ol (HP 029) analogues: acetylcholinesterase and amine uptake inhibitory properties

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Structure-activity relationships for the inhibition of cholinesterase and noradrenaline (NA) reuptake are shown in Table 1 with THA included for comparison. In general, compounds that are unsubstituted at the 9-amino group showed good cholinesterase inhibition. Of these, the unsubstituted parent compound (HP 029) was moderately potent (IC50 4.8 μM), while the 6-chloro derivative (compound 2) exhibited the strongest inhibition (IC50 0.01 μM). In marked contrast, the 7-chloro analogue (compound 4) was a very weak AChE inhibitor. The addition of a small alkyl group at the 9-amino position gave, in the case of the methyl derivative, a compound retaining good activity (AChE IC50 of 1.8 μM). When aryl substituents were attached to the 9-amine function by a -CH2- linkage (benzyl analogues), there was a marked decrease in potency (compounds 7, 9, 10 and 11). If the aryl group was linked to the 9-amino function by an alkyl chain longer than -CH2- (compound 8), good AChE inhibition was observed (IC50 3.4 μM). The primary amines (THA, HP 029, compounds 2, 3, and 4) showed essentially no inhibition of NA uptake. Substitution of the 9-amino group with small alkyl chains had no effect, whereas attachment of any alkyl group led to respectable NA uptake inhibition (IC50 < 1.8 μM). The phenethyl analogue (compound 9) and the 6-chloro substituted benzyl derivative (compound 12) had moderate anticholinesterase activity coupled with good NA uptake inhibition.

The present work extends previous studies with HP 029. As an AChE inhibitor, HP 029 is somewhat less active in vitro than THA and yet is as active in reversing memory deficits in cholinergically impaired animals (Shutske et al., 1988). Thus, compounds with weak-to-moderate anticholinesterase properties, as well as the most potent AChE inhibitors of Table 1, were considered for further investigation. THA, for example, has recently been reported to have ion-channel effects (Drukarch et al., 1987, Schaaf & Sattin, 1987) that may increase the release of endogenous neurotransmitters. Such effects could contribute to the usefulness of THA-like compounds in AD. The effects of halogen substitution on the acridine nucleus are also worthy of additional study. Whereas THA and HP 029 are more potent as inhibitors of BChE than AChE (Table 1; Heilbronn, 1961), halogen substitution at the 6-position of the aromatic ring, in addition to enhancing AChE inhibitory activity, reversed the selectivity of inhibition (i.e. AChE inhibition > BChE).
A most interesting observation was that several 9-amino benzyl substituted analogues were also potent inhibitors of biogenic amine uptake. The inhibition of NA uptake by compounds 7 (HP 128), 9, 10 and 12 occurred at substantially lower concentrations than the reported ion channel blockers. The most potent compounds were benzyl derivatives, followed by tricyclic and phenethyl-benzyl substituted analogues. This suggests that the inhibition of NA uptake is due to the presence of a benzyl group in the molecule.

DNA damage and repair in Alzheimer's disease lymphocytes

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Little work has been done on DNA repair in Alzheimer's disease (AD) cells, but several recent studies have indicated that these cells, like Down's syndrome lymphocytes, may be more sensitive to DNA damage than normal lymphocytes. We recently examined DNA repair after γ-irradiation in lymphocytes from AD and from normal individuals by measuring unscheduled DNA synthesis (UDS). We have now examined DNA replication and rate of rejoining of single-strand breaks in these cells after irradiation.

For replication studies, lymphocytes were γ-irradiated with 1.25 or 3.25 Gy and after 4 h, a known number of cells was stimulated with phytohaemagglutinin (PHA) and then maintained in culture medium; 66 h later they were incubated with [3H]thymidine for 1 h. Triplicate measurements were made on most samples. In some cases, the number of viable cells was counted just before addition of [3H]thymidine so that the incorporation per cell numbers present (as opposed to the incorporation per initial number of unstimulated cells) could be calculated.

In unirradiated lymphocytes, [3H]thymidine incorporation was about 40% lower into AD cells than into age-matched normals; also, there was a slight, but not statistically significant, decrease in uptake with age into normals. However, incorporation into irradiated, stimulated cells, expressed as a percentage of the uptake into unirradiated cells, was similar for AD patients, normal age-matched individuals and young and old normals.

These results indicate that after irradiation, DNA synthesis is quantitatively similar in those AD cells and normal cells, from donors of various ages, that survive for at least several days after treatment. As to unirradiated cells, it appears that fewer AD than normal lymphocytes are stimulated by the mitogen, i.e. there may be an abnormality of T-cell function in AD.

To investigate initial damage and initial rate of repair after irradiation, we examined the size of the DNA in PHA-stimulated lymphocytes at various times after irradiation. The DNA was radiolabelled by incubation of cells with [3H]thymidine, usually 50 h after stimulation (we checked that the level of [3H]incorporation was not such as to cause strand-breaks). Cells were then irradiated with 150 Gy, lysed on alkaline sucrose gradients and centrifuged at 18°C for 2 h at 29 000 rev./min (Spinco SW41Ti rotor, Beckman ultracentrifuge). After fractionation of gradients by upward displacement, DNA peaks were located by scintillation counting of each fraction. Values of average molecular mass reveal any differences in rate of repair, a 15 min period was tried also; however, no difference was found between values of the benzyl derivatives were more potent than the tricyclic antidepressant, desipramine, (Meyerson et al., 1980) as biogenic amine reuptake inhibitors. Compounds with combined cholinomimetic and adrenergic properties may be more efficacious for the treatment of multiple neurotransmitter deficits in AD.


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