Tacrine, a Drug with Therapeutic Potential for Dementia: Post-Mortem Biochemical Evidence

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ABSTRACT: A review of biochemical findings is presented which support the idea that Alzheimer's disease represents a condition for which tetrahydroaminoacridine (tacrine) may have a beneficial effect. There is evidence that clinical and histopathologic hallmarks of the disease relate to cholinergic and serotonergic dysfunction, with less obvious abnormalities in other neurotransmitters (aspartate, dopamine, gamma-aminobutyrate, glutamate, noradrenaline and somatostatin). Clincially relevant concentrations of tacrine may ameliorate the above presynaptic deficits without producing harmful (neurotoxic) effects of aspartate and glutamate. The disease seems to be associated with an early and clinically relevant degeneration of some neurons with cortical perikarya that release these amino acid transmitters. Studies are now required on the effect of tacrine on postulated harmful peptide-bond hydrolase activity within and around such cells.

RÉSUMÉ: La tacrine, un médicament ayant un potentiel thérapeutique dans la démence: évidence biochimique post-mortem Nous présentons une revue des observations biochimiques supportant le concept que la maladie d'Alzheimer représente une pathologie dans laquelle la tetrahydroaminoacridine (tacrine) peut avoir un effet bénéfique. Il existe des données qui laissent croire que les stigmates cliniques et histopathologiques de la maladie sont en relation avec une dysfonction cholinergique et sérotoninergique, accompagnée d'anomalies plus discrètes des autres neurotransmetteurs (aspartate, dopamine, gamma-aminobutyrate, glutamate, noradrénaline et somatostatine). Des concentrations cliniquement appropriées des tacrine peuvent améliorer ces déficits présynaptiques sans produire les effets noscifs (neurotoxiques) de l'aspartate et du glutamate. La maladie semble être associée à une dégénérescence précoce et cliniquement significative de certains neurones, dont le corps neuronal est cortical, qui libèrent ces acides aminés agissant comme transmetteurs. L'effet de la tacrine sur l'activité de l'hydrolase, agissant au niveau des liaisons peptidiques et qu'on postule être néfaste dans et autour des cellules, doit maintenant être étudiée.

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Recent data[1] (also unpublished observations) indicate that clinically relevant concentrations of 1,2,3,4-tetrahydro-9-aminoacridine (tacrine) are unlikely to produce harmful (neurotoxic[2]) effects of the excitatory amino acids (EAA, aspartate and glutamate). Such systemic doses, however, influence brain concentrations of other transmitters affected in Alzheimer's disease (AD). Data indicate that, at low concentrations, tacrine modulates monoaminergic transmission and inhibits acetylcholinesterase.[3,4] It seems likely, therefore, that any effect of tacrine in AD depends, at least in part, upon its action on components of cholinergic and monoaminergic systems. We present here a review of postmortem biochemical findings which support the idea that AD represents a condition for which tacrine may have a beneficial effect.

Problems Associated with Postmortem Neurochemical Studies

The prospect of obtaining meaningful data from diseased postmortem brain may be questioned as parameters under scrutiny may be influenced by factors such as patient age, sex, drug history, immediate pre-mortem status (sudden death or prolonged coma) and postmortem delay. Interpretation of postmortem data therefore requires fastidious consideration of these factors in order to separate changes that are due to the brain disease from those that occur as a result of epiphenomena.[5-9]

Tissue atrophy is another factor which may confound interpretation of data. This is because the practice of reporting results relative to unit mass (e.g. per mg of protein) does not make allowance for any reduction in volume of brain structure.

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Shrinkage or loss of some structures, but not others, may lead to the reporting of an increase in the markers of the unaffected structures, and, perhaps more importantly, an apparently small decrease in markers of the affected structures. For example, if a 35% reduction occurred of a structure constituting two-thirds of tissue volume, the reported loss of a biochemical marker (absent from unaffected structures) would be just 15%. Thus, loss of a biochemical marker may be considerably underestimated, should the structure to which it relates represent a large proportion of tissue volume, such as pyramidal neurons within the cerebral cortex. Transmitter glutamate, probably associated with these pyramidal neurons of the cerebral cortex, may account for only 10-30% of tissue glutamate[9,10] so even major losses of this structure would be difficult to detect from the glutamate content. Should losses occur equally in all cellular components (e.g. of pyramidal neurons and associated structures organized in columns), there would be no reported change in biochemical measures, even in severely atrophied tissue. Only a few studies have attempted to make allowance for this factor, by expressing results per entire brain region.[11-13] Thus, although a hypertensive-agent related enzyme (angiotensin-converting enzyme) may be increased in AD,[14,15] the change in mixed senile and vascular dementia is more obvious when allowance is made for atrophy.[12] Likewise, the extent of loss in AD of serotonin (5-HT) from the frontal lobe is only evident with such correction.[13]

It is not possible to distinguish reliably whether a reduced concentration of a marker is due to loss of the neurons which normally possess it, or to an alteration in the rate of its turnover, which may be a secondary phenomenon. Sodium-dependent uptake and calcium-dependent release of transmitter (depolarization evoked) provide important markers for the study of brains from experimental animals. However, because both of these processses require the maintenance of membrane potentials, they cannot be examined postmortem unless tissue is obtained shortly after death and assayed immediately[9,16]; tissue frozen in isotonic medium has also been used.[17] Postmortem changes also influence the activity of specific marker enzymes such as dopamine-beta-hydroxylase and tryptophan hydroxylase. Finally, it is difficult to identify changes occurring early in AD by examination of tissue obtained postmortem, where the disease has usually run its full course.[18]

Neocortical Neurons

Excitatory amino acids (pyramidal neurons)

Pyramidal cells are the largest and most abundant neuron type in the cerebral cortex. Recent quantitative morphological data on the distribution of plaques and tangles suggest that AD may result at least, in part, from a loss of structural and functional integrity of pyramidal neurons forming corticocortical association projection fibres.[9,19]

The integrity of pyramidal neurons has been difficult to assess biochemically because of the ubiquitous distribution and many functions of EAA's. However, postmortem studies of both the uptake and binding of radiolabelled D-aspartic acid suggest that loss of some terminals of EAAergic neurons occurs, though the magnitude and distribution of such change, the extent of influence of artefact and epiphenomena and the location of the cell bodies is not yet clear.[9,11,20-25]

Gamma-aminobutyric acid (GABA)

Immunohistochemical studies have indicated that the majority of non-pyramidal interneurons in the cortex stain with antisera against GABA or its biosynthetic enzyme glutamic acid decarboxylase (GAD). Thus, GABA constitutes a major inhibitory transmitter system in the cortex, accounting for as many as 30 per cent of all cortical neurons.[26] Large reductions in cortical GAD activity in AD were originally published alongside some of the first reports of diminished choline acetyltransferase (ChAT) activity. However, detailed studies of brain tissue obtained from diagnostic craniotomies and the brains of experimental animals suggested that loss of GAD activity was attributable to the terminal hypoxia associated with protracted death.[7] No change in GAD activity was found in a recent study where AD and control subjects were carefully matched for agonal state.[27] Surprisingly, concentrations of GABA in subcortical structures have not been shown to be similarly affected[5] although GABA concentrations in the parietal cortex are probably affected by agonal state.[7] There is evidence of reduced GABA concentrations in the temporal, frontal, parietal and occipital lobes in AD.[7] The reductions found in most studies are less substantial and widespread than those reported by Ellison and colleagues[28] who assayed subjects of similar age but included only pathologically severe examples of the disease. Large and widespread reductions in uptake sites of GABAergic neurons have also been reported.[29] However, since this was based upon active uptake determinations in tissue that had been frozen, thawed and subfractionated it is difficult to exclude the possibility that inappropriate preparations were produced in disease affected tissue. Indeed, Simpson and colleagues[30] find preservation of this uptake site (assayed using a ligand binding technique) in all regions examined, except the temporal cortex.

Neuropeptides

These appear to be localized in one broad class of interneuron, forming approximately 5% to 10% of the total cortical cell population. Current information indicates that virtually all of these interneurons are GABAergic.[26,31] Neuropeptides have been demonstrated to be stable in postmortem tissue and so they have been extensively studied in AD.[32]

Despite relatively high concentrations in cerebral cortex, cholecystokinin, vasoactive intestinal polypeptide and neuropeptide Y are unaffected. This may be adduced as evidence of relative sparing of the neurons that contain these peptides. The normal concentrations of vasoactive intestinal peptide are of particular interest in view of the reported co-existence between this peptide and ChAT activity within a population of non-pyramidal bipolar neurons. Such co-existence is not found in ascending cholinergic projections so the sparing of the peptide in AD may also indicate a sparing of intrinsic cholinergic neurons. Galanin exists in human basal forebrain cholinergic neurons but its "overactivity" may accentuate cholinergic dysfunction.[33] The localization of corticotropin-releasing factor in the cerebral cortex is not clear but it has been reported reduced,[34,35] whereas galanin appears unaltered.[36]

Many studies have demonstrated reduced somatostatin-like immunoreactivity (SLIR). Changes have been found to be greater in studies where only subjects displaying severe histopathology were examined than in those where no such

selection criteria were employed.[7,37] A difference in selection criteria may also explain why Beal and colleagues[38] found reduced concentrations of neuropeptide Y whereas other groups have reported no change. A proportion of SLIR neurons in human cerebral cortex also stain for neuropeptide Y,[39] so it is difficult to reconcile loss of SLIR with the sparing of neuropeptide Y. Studies in biopsy specimens from AD patients have not revealed any significant abnormality in the content or release of SLIR.[37]

Corticopetal Neurons

Acetylcholine

Evidence for presynaptic cholinergic dysfunction in the cerebral cortex in AD has been obtained in all postmortem studies that have measured ChAT activity, radiolabelled choline uptake[40] or acetylcholine content and release.[41,42] These data are supported by a myriad of histological studies that have reported loss of cholinergic perikarya from the medial forebrain nucleus basalis of Meynert.[43] Reduced ChAT activity is also a feature of "senile dementia of the Lewy body type"[44] (E.K. Perry, personal communication).

Serotonin

Serotonergic neurons, like cholinergic cells, innervate large areas of cerebral cortex from discrete extra-cortical (raphe) nuclei. However, serotonergic activity has been less thoroughly investigated in AD, largely because of the difficulty of measuring the activity of tryptophan hydroxylase in postmortem brain. With the exception of studies of the 5-HT carrier,[45-47] all estimates of serotonergic neurons in postmortem samples have relied upon determination of the concentrations of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA[27,47,48]). The 5-HT content in the neocortex from AD subjects has in general been found to be reduced whereas 5-HIAA was unaltered except for two reports of a reduced content of this metabolite.[2-7,49] This discrepancy may be related to postmortem delay which was shorter in the latter studies than in studies of large groups of samples.[48] There is some evidence from histopathologic measurements to indicate that intrinsic cortical change and serotonergic denervation are related since significant negative correlations were found between tangle counts and 5-HIAA content in both frontal and temporal cortex of AD subjects.[48] Aggressive AD patients have a particularly low fronto-cortical concentration of 5-HT, suggesting that treatment with serotonomimetic agents could reduce the need for hospitalization.[13]

Catecholamines

The study of catecholaminergic neurons has been hampered by the postmortem instability of the enzymes responsible for the synthesis of dopamine (DA) and noradrenaline (NA). Apart from two studies of dopamine-beta-hydroxylase activity postmortem, one indicating reduced activity and other finding no change, biochemical studies of the cerebral cortex have focussed upon the determination of the concentrations of DA and NA and their principal metabolites, homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), respectively. The concentration of DA has consistently been found not to be reduced.[48] HVA concentrations are reported reduced in some regions, but elevated in others.[48] Reduced concentrations of NA have generally been reported[47,48] whereas concentrations of MHPG have been found to be reduced, unaltered or even elevated.[27,48] This may be a reflection of the postmortem accumulation of MHPG,[8] which, together with the high turnover rate and low concentrations of catecholamines in the cortex, make questionable the validity of determination of tissue concentrations postmortem. Moreover, since oxygen is a cofactor of both tyrosine hydroxylase and dopamine-beta-hydroxylase, the terminal hypoxia usually associated with AD may also be partly responsible for some of the observed changes. Noradrenergic changes in the cortex may be associated with neuronal loss from the brain stem noradrenergic nucleus, the locus ceruleus.[50]

Neurotransmitter Recognition Sites

Ligand binding studies in AD have indicated that some neurotransmitter recognition sites are *unaltered* in the cerebral cortex, even with allowance for tissue atrophy.[14] This includes those for a catecholamine, opiates, GABA and acetylcholine (muscarinic type). Assay of muscarinic receptor subtypes[51] show the high affinity pirenzapine binding site (M_1 receptor), the major postsynaptic receptor, to be apparently preserved,[52,53] although no allowance for any atrophy was made. These findings suggest that a suitable target for acetylcholine is available in the cortex of AD patients but successful therapy with a cholinomimetic would help prove that this receptor is functionally intact.[54] M_2 muscarinic receptors and nicotinic receptor sites (both of which are present in smaller numbers and may be primarily presynaptic) are reduced in the disease.[42,52,53,55] Further studies on the location and physiological role of these receptors are necessary to assess the significance of these losses. Another consistent abnormality is that of recognition sites for 5-HT. Binding of [^{3}H]5-HT (to the 5-HT_1 site) has been shown to be significantly reduced in the areas of cortex examined. Lower binding of [^{3}H]ketanserin (to the 5-HT_2 site) has also been described.[11,56] The binding of [^{3}H]lysergic acid diethylamide (to both 5-HT_1 and 5-HT_2 sites) is reported significantly reduced in most studies, including the only one to make allowance for tissue atrophy by examination of the entire temporal lobe.[14,45] 5-HT_{1A} receptors are reduced in some areas.[57,58]

Although altered populations of EAA recognition sites have been reported in the neostriatum and the hippocampus, the neocortex has shown few alterations.[19] Lower glutamate binding in cortical layers 1 and 2 and reduced glycine modulation of the N-methyl-D-aspartate (NMDA) receptor have been described[19,59] (the reduced binding of [^{3}H]-TCP to poorly washed membranes[25] may also reflect this latter change). Reduced binding of radiolabelled somatostatin[60] and corticotropin-releasing factor[35] have been reported in the cerebral cortex, together with loss of DA D_2 receptors from the neostriatum, with the latter possibly reflecting loss of *aspartate-releasing corticofugal pyramidal neurons* from this region.[8,61,62,63]

Data from Biopsy Samples

Small amounts of AD brain tissue, removed for diagnostic purposes,[64,65] have also been used for biochemical analyses,[66] an approach pioneered by Korey and colleagues.[67] The few samples offered to the authors (39, between 1976 and 1987) originated from 7 centers. The material has, however, contributed to knowledge of the biochemistry of AD. Firstly, it has

helped to separate disease-related change from the artefact and the epiphenomena normally associated with postmortem tissue (e.g. problems associated with GAD activity measurements). Secondly, it has allowed firmer conclusions to be drawn about neuronal integrity by permitting assessment of a variety of biochemical markers for a single neuronal type (Table 1). Thirdly, it has provided some insight into the neuronal changes that occur early in the disease, and, together with postmortem data, changes that occur at a later stage (Table 2). Finally, biochemical measures assessed antemortem in this laboratory have the advantage that there has often been a fixed and short duration (<2 weeks) between neuropsychological assessment and the removal of tissue.[64,65] Moreover, detailed clinicopathological assessments have been made, including a rating of the magnitude of dementia on the basis of the performance of patients on a number of tests that assessed the extent of the following clinical domains: memory, perceptuo-spatial abilities and language. The rating correlated with acetylcholine synthesis,[65,73] but not with other corticopetal transmitters[18] and GABA was apparently increased or unaltered.[7] Loss of pyramidal neurons may be a critical change since atrophy-corrected counts of pyramidal cells in cortical layers III and V of the same subjects were found to correlate with the magnitude of dementia.[65] The significant association between PET-determined ^{18}F-deoxyglucose uptake and behaviour[74,75] may also reflect a relationship between pyramidal cell dysfunction and the clincial symptoms of AD.

The fact that the disease is associated with alterations in potential biochemical markers of *glutamate-releasing cortico-cortical pyramidal neurons*, (Table 2), and structural changes in the cerebral cortex are demonstrated by other techniques (above), indicates a role for these cells in pathogenesis. For example, the postmortem data indicating a relationship between tangle counts and the cortical concentration of 5-HIAA have been confirmed in antemortem tissue, suggesting a pathogenic link between cortical cell dysfunction and corticopetal fibers.[70] Retrograde degeneration[76] of corticopetal cholinergic fibers (also from the cholinergic septal-hippocampal pathway) may occur as a consequence of reduced output of neurotrophic factors by the cortex (e.g. nerve growth factor[77,78]). Hypotrophic activity is likely to be secondary to the insult that causes death of EAA-releasing (see text and the seminal study of Hyman and colleagues[79]) corticocortical, corticofugal and hippocampal pyramidal neurons and the accumulation within these cells of abnormal inclusions (tangles, possibly granulovacuolar degeneration and Hirano/Lewy body formation.) The *insult* to these neurons is ascribed here to a toxin/infectious agent[80,81] inducing alterations in energy metabolism[9,82-84] leading to a reduced pH value within and around pyramidal cells and stabilization of neutral-pH labile peptide-bond hydrolases.[85] Owing to the protracted course of the disease, only a small portion of the brain

Table 1: Summary of Neurotransmitter Measures Assessed Antemortem in Neocortex of Patients with Alzheimer's Disease

		AD as % of Control	Reference
Cortical perikarya			
-GABA			
	Release*	NS	68
	Concentration	NS	7
	GAD activity	NS	69
-Somatostatin			
	SLIR release*	NS	37
	SLIR concentration	NS	37
-EAAs			
	Aspartate release*	NS	68
	Glutamate release*	NS	68
	Aspartate concentration	124	9
	Glutamate concentration	86	9
Subcortical perikarya			
-Acetylcholine			
	Synthesis	41	70
	ChAT activity	35	70
	Choline uptake	57	71
-5-HT			
	Release	51	70
	Concentration	31	70
	5-HIAA concentration	44	70
	Uptake	39	70
-NA			
	Release	NS	18
	Concentration	32	18
	MHPG concentration	NS	18
	Uptake	47	18
-DA			
	Release	NS	18
	Concentration	NS	18
	DOPAC concentration	NS	18
	HVA concentration	NS	18

Percentages are given only for values that were significantly different from control; N.S. not significant; *static incubations without a reuptake inhibitor.

Table 2: Excitatory Amino-Acid Neurotransmission in the Neocortex from Alzheimer Subjects Some 3 Years (neurosurgical samples) and 6-10 Years (autopsy material) After Onset

	Early stage	Late Stage
Glutamate,		
- concentration	decreased	decreased*
- release	unchanged	nd
Aspartate,		
- concentration	increased	unchanged
- release	unchanged	nd
Glutaminase activity	unchanged	decreased**
D-aspartate uptake	nd	decreased*
D-aspartate "binding"	nd	decreased**
NMDA-phencyclidine receptor		
- cation channel	nd	unchanged
- glutamate site	nd	unchanged
- glycine site	decreased	decreased
- "zinc site"	nd	unchanged
Kainate receptor binding	nd	unchanged
Quisqualate receptor binding	nd	unchanged***

decrease/increase, identifies significant changes; nd, not determined; * specimens obtained within 3h of death ** subject to unexplained variability or artefact *** hippocampus (see Table 1; also references 9, 11, 19, 24, 59, 67, 72, and unpublished observations).

may show these changes at any given moment.[86] It has been suggested that these changes begin in olfactory areas of the brain. The subsequent spread is via well defined neuronal pathways, initially involving cells of the limbic system but, as the disease progresses, those of the neocortex.[57,76,87] Another mechanism proposed for increased protein degradation[88] is proteolytic activity associated with cholinesterase-rich pyramidal neurons.[89,91]

It is well known that tacrine is an effective inhibitor of acetylcholinesterase and a preliminary study[92] has tentatively confirmed the reported therapeutic efficacy in AD.[3] Studies are now required on the effect of tacrine on the postulated harmful proteolytic activity within and around pyramidal cells, in particular in relation to the turnover of cell survival-related receptors[2,93,94] as well as nerve growth factor. There is now some hope that disease progression[95] can be slowed down by an inhibitor of peptide-bond hydrolases so it is particularly important to determine *rate of decline* in cognitive performance,[96] before inclusion in a clinical trial. Clearly, improved methods of early diagnosis are urgently required.

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