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Review

Is there a rationale for the use of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease?

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Abstract

Since the 1980s, the cholinergic hypothesis of the pathogenesis of Alzheimer's disease has proven to be a strong stimulus to pharmacological strategies aimed at correcting the cognitive deficit by manipulating cholinergic neurotransmission. Among these strategies, the one based on acetylcholinesterase inhibition is currently the most extensively developed for the therapy of Alzheimer's disease. The inhibitors' mechanisms of action are complex, including changes in the release of acetylcholine, and modulation of acetylcholine receptors. Various clinical trials of various inhibitors have shown that, on the whole, their effects were modest and, in the case of some drugs, were associated with frequent adverse reactions. Among the conceivable reasons for the limited efficacy of these drugs, those related to the pharmacological target deserve particular attention. This review, therefore, focuses on the complex nature of the acetylcholine system, the alterations of acetylcholinesterase and muscarinic receptor signal transduction in Alzheimer's disease, and the involvement of other neurotransmitters. © 1998 Elsevier Science B.V.

Keywords: Acetylcholinesterase inhibitor; Alzheimer's disease

1. Introduction

Alzheimer's disease, the most common cause of dementia in the elderly, is a chronic, slowly progressive neurodegenerative disorder with characteristic deterioration of intellectual capacity in various domains: learning and memory, language abilities, reading and writing, praxis, interaction with the environment. Personality changes are common, leading to behavioural disturbances. Patients lose the ability to perform activities of daily living. In later stages, basic biological functions are disturbed and neurological alterations develop (Tariot, 1994).

Because of the serious functional impairment, the high cost of care and the emotional impact on affected families, it is not surprising that there is enormous pressure to develop effective therapies.

The physiopathology of Alzheimer's disease is complex. Severe neuron and synapse loss, neurofibrillar tangles and senile plaques are prominent in Alzheimer's disease. The tangles represent the accumulation of abnormal components of the neuronal cytoskeleton aggregating into paired helical filaments, whereas the plaques are composed of dystrophic neurites and glial elements and have a core of β-amyloid peptide, which is derived from the larger β-amyloid precursor protein (APP). There is evidence pointing to the production and deposition of β-amyloid as critical events in the pathogenesis of Alzheimer's disease (Cummings et al., 1996; Forloni et al., 1996; Selkoe, 1994; Yankner, 1996), although this point is somewhat controversial (Davis and Chisholm, 1997).

Since the early 1970s, highly consistent findings have clearly shown that some neurotransmitter systems are selectively altered in the Alzheimer's disease brain. The most dramatic abnormalities are those of the cholinergic system, providing the foundation for the so-called 'cholinergic hypothesis of Alzheimer's disease'. Since the aetiology and exact pathogenesis of Alzheimer's disease were (and still are) unclear, this hypothesis formed the rationale for a symptomatic therapy of Alzheimer's disease aimed at potentiating central cholinergic function, in the hope that this would improve cognitive function.

Based on this strategy and beginning in the 1980s, a number of compounds were studied in preclinical models

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Table 1
Some acetylcholinesterase inhibitors that are marketed or under clinical evaluation for the treatment of Alzheimer's disease

Drug	Company	Clinical phase
Tacrine	Warner-Lambert	IV
Donepezil	Eisai/Pfizer	IV
Eptastigmine	Mediolanum	III
ENA-713 (Exelon™)	Novartis	III
Galanthamine	Shire Pharm.	III
Metrifonate	Bayer	III
Physostigmine slow release	Forest	III
Huperzine A	Chinese Acad. Sciences	III
TAK-147	Takeda	III
NIK 247	Nikken	III
CP-118954	Pfizer	II
MDL 73745	Marion Merrel Dow	II
NX-066	Astra Arcus	II

From Giacobini (1996), mod. Due to the rapidly expanding area, this list and the clinical phase might now be outdated.

of cognitive impairment and subsequently tested in clinical trials. As a result of this extensive effort, two inhibitors of acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) were approved in the USA and other countries, i.e., tacrine (1993) and donepezil (1997). Numerous other compounds are currently under clinical development (Table 1).

So far, however, this strategy appears to be only modestly effective.

The aim of this review is to provide a critical reappraisal of the current status of Alzheimer's disease therapy. The following issues will be addressed:

–the cholinergic hypothesis of Alzheimer's disease;
–the acetylcholinesterase inhibitors currently used or tested for the therapy of Alzheimer's disease, their mechanism(s) of action, the clinical trials;
–the possible reasons for their limited efficacy in Alzheimer's disease.

2. The cholinergic hypothesis of the pathogenesis of Alzheimer's disease

The physiologically ageing brain has long been known to show alterations of cholinergic function and cognitive impairment (reviews by Muir, 1997; Müller et al., 1991).

The cholinergic hypothesis of Alzheimer's disease stems from the following findings at post-mortem and biopsy studies (Bowen et al., 1977; Davies and Maloney, 1976. Reviews by Bartus et al., 1982; Coyle et al., 1983; Giacobini, 1990; Palmer and Gershon, 1990; Pepeu, 1988; Perry, 1986):

(i) deficits in acetylcholine synthesis and in the activity of choline acetyltransferase and high-affinity choline uptake;
(ii) reduced acetylcholine release and metabolism (acetylcholinesterase activity);
(iii) the loss of nicotinic, but not muscarinic receptors.

A comparison between biopsic and autopsic cortical samples indicated the early appearance of these lesions (DeKosky et al., 1992). Although these neurochemical findings could be affected by a number of factors including the end stage of the disease, the therapy, the preterminal state and the post-mortem delay and the tissue storage conditions, they were corroborated by the results of several morphometric, histopathological and neuroimaging studies (reviewed by Foster, 1994; Geula and Mesulam, 1994).

The cholinergic deficit in Alzheimer's disease results from extensive cell loss especially in the neocortex, the amygdala and the hippocampus. The nerve terminals in these areas originate from the cholinergic basal forebrain system, i.e., the medial septum, the diagonal band of Broca and the nucleus basalis of Meynert. Severe neuronal loss was reported for practically all these nuclei, especially the nucleus basalis, consistent with the marked loss of choline acetyltransferase activity in the cortical areas that are primarily innervated by these neurons (review by Höhmann et al., 1988).

The relevance of the alterations of the cholinergic system in Alzheimer's disease was further substantiated by the finding that cholinergic neurotransmission modulates learning, memory and attention, as shown by: (i) the correlation between cortical choline acetyltransferase deficit, senile plaques and cognitive impairment in Alzheimer's disease patients (reviews by Geula and Mesulam, 1994; Mountjoy, 1986); (ii) the cognitive deficit induced by centrally active anticholinergic compounds (e.g., scopolamine) both in normal humans and experimental animals, that is somehow reminiscent of the deficit in Alzheimer's disease (review by Hagan and Morris, 1988); (iii) the learning and memory impairment in experimental animals that is associated with the degeneration of the basal forebrain induced by electrolytic lesions or with excitotoxins, cholinotoxins or immunotoxins (reviews by Baxter and Gallagher, 1997; Muir, 1997). A similar impairment was found in transgenic mice overexpressing human acetylcholinesterase (Beeri et al., 1995).

Since the early 1980s, the strategies for manipulating the cholinergic systems and inducing learning and memory disorders in animals have been applied to the study of new drugs potentially capable of reversing these alterations and improving the animal's cognitive abilities. It was long, and generally accepted that such drugs could eventually be applied in the treatment of Alzheimer's disease. However, the predictive value of these tests has been questioned and this controversy is reinforced by the only modest clinical efficacy of experimentally active drugs (reviews by Fibiger, 1991; Hanin, 1996; Holttum and Gershon, 1992; Pepeu, 1994; Sarter et al., 1992a,b). Clearly, these models do not address the fundamental issue of the neuropathological causes of Alzheimer's disease.

Recently, more relevant animal models have been developed. The injection of β-amyloid into the rat brain induced amyloid deposit, neurotoxicity, cholinergic hypofunction and disruption of memory processes (Frautschy et al., 1996; Giovannelli et al., 1995). Animal models of neurodegeneration have improved much since the development of transgenic animals expressing the human gene for the APP, or some portion of it (see Special Issue of Neurobiol. Aging, 1996). In some of these mice, β-amyloid deposition and neuropathological alterations were associated with an age-dependent deficit in spatial learning (Hsiao et al., 1996; Moran et al., 1995; Nalbantoglu et al., 1997).

Hopefully, there will be a reproducible, cognitive and behavioural deficit that can be linked to the primary neuropathological features of Alzheimer's disease. Once made available to the scientific community, this would represent a major step forward in the production of an animal model in which potentially new therapies could be evaluated.

3. Acetylcholinesterase inhibitors for the therapy of Alzheimer's disease

The cholinergic hypothesis of Alzheimer's disease has provided the rationale for the current major therapeutic approach to Alzheimer's disease. This approach is based on the attempts to correct the cognitive decline by manipulating cholinergic neurotransmission.

Of the various possible strategies for enhancing cholinergic activity in the brain, acetylcholinesterase inhibition has been by far the most extensively used. The acetylcholine receptor agonists provide a possible alternative, the development of which, however, is still lagging behind (review by Weinstock, 1995).

The inhibitors of acetylcholinesterase should increase the efficiency of cholinergic transmission by preventing the hydrolysis of released acetylcholine, thus making more acetylcholine available at the cholinergic synapse. Like acetylcholine receptor agonists, acetylcholinesterase inhibitors are assumed to take advantage of the relative preservation of post-synaptic muscarinic receptors in Alzheimer's disease (Giacobini, 1990).

Among these compounds, tacrine and physostigmine have been the most extensively studied for their ability to inhibit the acetylcholinesterase.

3.1. Mechanism(s) of action of acetylcholinesterase inhibitors

In general, these compounds can be divided into three main classes on the basis of their structure and mechanism: (i) *tertiary amino compounds* (reversible inhibitors either non-competitive, like donepezil, or of mixed type, like tacrine, which causes allosteric inhibition of acetylcholinesterase by binding to a hydrophobic region near the anionic α or β sites on its surface), (ii) *carbamates* (e.g., ENA 713 and eptastigmine, pseudo-irreversible inhibitors which form a carbamoylated complex with the serine residue of the catalytic triad of the enzyme that is hydrolyzed at a slower rate than the acylated form), and (iii) *organophosphates* (irreversible inhibitors like dichlorvos, the active metabolite of metrifonate, which forms a covalent bond with the serine residue, thereby giving rise to a stable enzyme-inhibitor compound). Moreover, some inhibitors are described as selective for acetylcholinesterase (e.g., donepezil, ENA 713, galanthamine), whereas tacrine appears to be a more potent inhibitor of butyryl-cholinesterase (Freeman and Dawson, 1991). Whether the selectivity for acetyl- vs. butyryl-cholinesterase results in low peripheral cholinergic effects in Alzheimer's disease patients has not been completely clarified.

It had been postulated that a therapeutic efficacy window of 30–60% of erythrocyte acetylcholinesterase inhibition exists, with a mid-point of 40% producing at least 50% clinical improvement (Becker et al., 1994). More recent animal experiments have shown an inconsistent relationship between the degree of acetylcholinesterase inhibition, the changes in brain acetylcholine concentration and improvement in cognitive function. This and other observations led to the proposal that the extracellular level of acetylcholine might be a better index of therapeutic activity (review by Giacobini and Cuadra, 1994).

Microdialysis studies showed a striking increase in acetylcholine cortical release in awake rats treated with physostigmine, eptastigmine, donepezil or metrifonate, though the extent of the effects was greatly variable (Giacobini et al., 1996; Messamore et al., 1993; Mori et al., 1995a; Scali et al., 1997). Tacrine showed mixed effects on acetylcholine metabolism (review by Wagstaff and McTavish, 1994). In rat brain slices, it inhibited acetylcholine release (which was enhanced by physostigmine and metrifonate), possibly by feedback inhibition of acetylcholine synthesis via stimulation of presynaptic receptors (Drukarch et al., 1987; Hallak and Giacobini, 1989). In vivo, tacrine (but not metrifonate) decreased high-affinity choline uptake and acetylcholine synthesis in the mouse brain at 3 mg kg^{-1} i.p., a dose that is comparable on a weight basis with the clinical doses (Nordgren et al., 1992). However, tacrine and physostigmine enhanced acetylcholine striatal release as assayed by microdialysis in anesthetized rats (Xiao et al., 1993).

It should be noted that a damaged cholinergic system, as in Alzheimer's disease, would benefit only if the acetylcholinesterase is inhibited to such a degree that the released acetylcholine can function post-synaptically without affecting the pre-synaptic events necessary for renewed transmitter synthesis and release (Nordgren et al., 1992).

As regards the receptors, binding studies showed that acetylcholinesterase inhibitors (especially tacrine) interact with both muscarinic (M_1, M_2) and nicotinic receptors

(review by Wagstaff and McTavish, 1994). The affinity of tacrine appeared to be approximately 100-fold greater for muscarinic receptors than for the nicotinic ones, both in the control and Alzheimer's disease cortex. Ex vivo, the number of muscarinic M_1 and M_2 receptors in the rodent brain was down-regulated following 14-day treatment with tacrine at 10 mg kg^{-1} day^{-1} s.c., but the number of nicotinic receptors was increased. Physostigmine also reduced the number of muscarinic receptors, but did not affect nicotinic receptors (Nilsson-Håkansson et al., 1990). Another study confirmed the down-regulation of muscarinic M_1 (but not M_2) receptors in the cortex of rats treated subacutely with tacrine (Flynn and Mash, 1989).

As mentioned in Section 2, a significant loss of nicotine binding and relative stability of muscarinic binding were found in various brain areas of Alzheimer's disease patients by functional neuroimaging (positron emission tomography and single-photon emission computerised tomography). As shown by Nordberg et al. (1997), tacrine (80 mg day^{-1} for 3 months) restored cortical [^{11}C]nicotine binding (an effect that was attributed to indirect stimulation of nicotinic receptors via the increased acetylcholine amount in the synaptic cleft), but decreased the binding capacity of [^{11}C]benztropine in the temporal cortex of one patient, reflecting down-regulation of muscarinic receptors, similar to that found in rats.

The possibility of pharmacological modulation of the nicotinic receptors in Alzheimer's disease patients suggested the potential for therapy with nicotinic agonists (Whitehouse and Kalaria, 1995). In vitro, nicotine protected cultured rat cortical neurons from the β-amyloid-induced neurotoxicity. This effect was mediated through the α_7-nicotinic subunit (Kihara et al., 1997). In a model system of a transfected fibroblast cell line expressing the nicotinic α_4/β_2 subtypes, the number of these binding sites was increased by treatment with tacrine and galanthamine at low concentrations ($10^{-6} - 10^{-7}$ M), but was reduced at higher concentrations. Donepezil only increased the receptors in a concentration-dependent way (Svensson and Nordberg, 1997).

The clinical relevance of the in vivo tacrine-induced rise in nicotine receptors in Alzheimer's disease patients is unclear. Nicotine seems to improve human attentional function rather than memory function (Muir, 1997). The effects of tacrine (and other acetylcholinesterase inhibitors) on muscarinic receptors in Alzheimer's disease deserve further investigation in more patients and with more specific ligands for the muscarinic receptor subtypes. Should the preliminary finding of down-regulation be confirmed, it would cast some doubt regarding the rationale for acetylcholinesterase inhibitors in Alzheimer's disease therapy.

3.2. Clinical trials on acetylcholinesterase inhibitors

A detailed examination of the therapeutic efficacy of the acetylcholinesterase inhibitors is beyond the scope of this review. However, brief comment is warranted. In these trials, various outcome measures were assessed. Briefly, global cognitive scales included the Alzheimer's disease Assessment Scale–Cognitive Subscale, ADAS-cog (score 0–70), and the Mini-Mental State Examination, MMSE (score 0–30). The Global Assessment of Change was evaluated by the Clinical Global Impression of Change (CGIC), the Clinician's Interview-Based Impression (CIBI), or the Final Comprehensive Consensus Assessment (FCCA). Functional scales were also analysed, such as the Activities of Daily Living (ADL) and the Instrumental Activities of Daily Living (IADL).

3.2.1. Tacrine

This drug has been studied in numerous clinical trials in thousands of patients (reviews by Davis and Powchik, 1995; Schneider, 1993; Soares and Gershon, 1995; Wagstaff and McTavish, 1994; Wolfson et al., 1997). Following the favourable results of the first, widely publicized, cross-over study (Summers et al., 1986) which, however, was the object of severe criticism (Food and Drug Administration, 1991, Interim Report), various trials were conducted with this design, but with mostly negative results. Indeed, no statistically significant differences from placebo were reported in eight out of nine trials, and the only positive one (Eagger et al., 1991) also encountered technical difficulties (inadequate washout period: see Davis and Powchik, 1995).

In response to criticism of the cross-over design, various studies using a parallel group design were carried out. Two small-scale trials (Maltby et al., 1994; Weinstein et al., 1991) were negative, but three large-scale multicentre trials showed significant improvement, or less deterioration, in tacrine recipients compared with those given placebo.

In the study by Davis et al. (1992), which included a pre-trial 'enrichment' phase, a probably inadequate washout period complicated the interpretation of results, which, on the whole, were not very impressive.

The two most favourable trials (Farlow et al., 1992; Knapp et al., 1994) reported a significant 4- or 5-point improvement of the ADAS-cog score in the patients receiving the highest dose of tacrine (80 or 160 mg day^{-1}, respectively) over placebo. In the Knapp et al. (1994) study, the proportion of patients who improved by ≥ 4 points after 30-week treatment rose from 25% in the placebo group to 30% with 80 mg day^{-1} tacrine and 40% with 160 mg day^{-1}. Similarly, responders in the CIBI scale rose from 18% (placebo) to 25% (80 mg day^{-1}) and 42% (160 mg day^{-1}). Results were highly significant for the 160 mg day^{-1} group. However, the results of the two trials differed regarding other secondary parameters, and were negative for the ADAS non-cognitive subscale which focuses on behaviour and mood problems. Finally, those patients who had participated in the 30-week high-dose study and were thereafter treated openly at doses > 120 mg day^{-1} and followed for a minimum of 2 years, were

less likely to enter a nursing home than patients receiving lower doses, and showed a trend to lower mortality (Knopman et al., 1996). On the basis of the data of these two pivotal studies, the Food and Drug Administration approved tacrine for marketing.

These results should be appraised in the light of the need to continue the treatment (the drug's action is temporary), and of the side-effects of tacrine.

The major adverse effect of tacrine is liver toxicity, as shown by elevated serum aminotransferases, which were reported as a delayed, asymptomatic manifestation in almost all clinical trials on the drug. According to a study on 2446 patients monitored weekly (Watkins et al., 1994), the alanine aminotransferase value was above the upper limit of the normal range in 50% of the patients, was greater than three times this limit (a level considered clinically significant and demanding interruption of the therapy) in 25% of the patients, and greater than 10 times the limit in 6%. The latter value is consistent with the hepatocellular necrosis observed in liver biopsies (review by Wolfson et al., 1997). Hypersensitivity responses also occurred in some patients as shown by granulomatous hepatitis or tissue and peripheral blood eosinophilia.

The tacrine-induced hepatotoxicity was reversible within 5–6 weeks after withdrawal. Following resolution of hepatotoxicity, rechallenge with the drug was possible.

The unpredictability and variable severity of side-effects make weekly monitoring of serum aminotransferase mandatory (Wagstaff and McTavish, 1994). As stated by Watkins et al. (1994), ''the marked interpatient differences in susceptibility make it likely that tacrine could produce life-threatening hepatotoxicity in some patients. Until the highly susceptible patients can be readily identified, the risk of severe hepatotoxicity should be weighed against the potential benefit of tacrine in each individual''.

Symptomatic cholinergic adverse effects (mainly gastrointestinal) are also more frequent in patients receiving tacrine than in those given placebo.

On the whole, the tacrine-induced adverse effects were the main cause for the drop-outs who represented about 40–60%, in the various trials. This incidence was higher with the 160 mg day^{-1} dose, the dose that ultimately showed the greatest difference from placebo for clinical efficacy.

Drug interactions: since clinical trials excluded patients who were taking drugs possibly interacting with tacrine, the safety of concomitant treatments was not thoroughly investigated. An interaction with cimetidine and theophylline was reported from other studies and was accounted for by the interaction with the same form of Cyt. *P*450, CYP1A2 (Wagstaff and McTavish, 1994; Watkins et al., 1994). Tacrine may also stimulate gastric secretion, with a resulting increase in gastric irritation and bleeding in patients receiving non-steroidal anti-inflammatory drugs.

The CYP1A2 converts tacrine to reactive metabolites that can bind covalently to cellular proteins, thus inducing cellular toxicity (Madden et al., 1993). This could explain the mechanism of tacrine-induced hepatotoxicity.

In conclusion, the efficacy of tacrine has been demonstrated, but it has equally been demonstrated that the drug has modest beneficial effects in some patients and a high side-effect profile. In a recent study at the Johns Hopkins Dementia Research Clinic, involving 162 Alzheimer's disease patients to whom tacrine treatment was offered, only 35 (21%) accepted and 22 (63% of the participants) continued its use beyond 3 months. The most common reason for refusing or stopping tacrine was that the small benefits did not outweigh the side-effects, the four-time-a-day dosing, and the nuisance of the weekly blood test (Lyketsos et al., 1996). This study emphasises the importance of the risk-benefit equation to patients and their families.

3.2.2. Donepezil

Three double-blind trials were carried out, but, regrettably, only one was published in extenso. The improvement in ADAS-cog and MMSE was significant with the daily dose of 5 mg, but was not very impressive, and the CGIC was unaffected (Rogers and Friedhoff, 1996). A preliminary communication reported that donepezil at 5 or 10 mg day^{-1} for 24 weeks produced a significant improvement in ADAS-cog and CIBIC-plus in 150 patients per dose (Nightingale, 1997, communication by the Food and Drug Administration; abstract by Rogers et al., 1996). In these patients, it was calculated that the dose of 10 mg day^{-1} delayed by 1 year a 1-point loss of ADL (on a 9-point scale) (abstract by Friedhoff and Rogers, 1997). Finally, in a long-term open study of 133 patients, donepezil 5 or 10 mg day^{-1} produced a 4-point improvement in ADAS-cog score compared with baseline. Subsequently, the ADAS-cog score gradually increased, indicating that donepezil does not prevent disease progression. After 2 years, a 4-point difference was still evident, although this was in comparison with a historical control group (abstract by Rogers et al., 1995).

The main side-effects were mild cholinergic symptoms, particularly gastrointestinal. They were more evident with the 10 mg day^{-1} dose. So far, no significant laboratory abnormalities, including hepatic ones, have been reported. The other advantage of donepezil is the once-a-day formulation.

In conclusion, the donepezil-induced improvement is modest and there is no evidence that the drug slows the progression of Alzheimer's disease by affecting neuronal viability. However, the lack of hepatotoxicity and the administration schedule facilitate its use.

3.2.3. Eptastigmine

Three phase II studies showed that the drug effect followed a U-shaped dose-relationship and the dose of 60 mg day^{-1} produced the greatest improvement over placebo (4-points of ADAS-cog) (Troetel and Imbimbo, 1997). The development of reversible neutropenia in two cases caused

the interruption of one of these trials. This effect was not reported from other studies.

A phase III, multicentre, long-term trial indicated a slight improvement at 45 mg day^{-1}. The effect was greater in a subset of more deteriorated patients. There was a similar incidence of adverse effects with placebo and eptastigmine, 30 or 45 mg day^{-1}, but the drop-out rate represented 18% of the patients given eptastigmine at 30 mg day^{-1} and 12% of those receiving 45 mg day^{-1}, compared with 7% of those on placebo (Canal et al., 1996).

3.2.4. ENA 713 (Exelon™)

So far, only a brief account of an early, double-blind study in 90 patients has been published (Anand et al., 1996). Its results indicate that, compared with placebo, the drug had some effects on ADAS-cog and improved the CIBI-plus in a larger number of patients given the dose of 4 mg day^{-1} (but not 6 mg day^{-1}). Cholinergic side-effects were mild to moderate (Sramek et al., 1996).

3.2.5. Galanthamine

The clinical data available to date are limited, but the following findings suggest that this drug is only moderately effective: a 3–4 point difference from placebo in the ADAS-cog; improvement of CGIC in a greater proportion of patients than with placebo (Rainer, 1997; Wilcock and Wilkinson, 1997). Cholinergic adverse effects were mild to moderate.

3.2.6. Metrifonate

Two double-blind trials were conducted in which the doses were titrated to reach and maintain 40–70% inhibition of the red blood cell acetylcholinesterase on the basis of the hypothesis that such an inhibition is predictive of significative improvement in cognitive function. The results of both trials (Becker et al., 1996; Cummings et al., 1997) were modest and did not confirm the initial assumption. However, cholinergic adverse effects were mild.

In conclusion, to date, the magnitude of symptomatic improvement with acetylcholinesterase inhibitors and the proportion of patients who responded have been modest, even with a high level of inhibition. It should also be noted that: (i) clinical trials generally compared drugs vs. placebo, thus making it difficult to objectively evaluate the efficacy of innovative drugs; (ii) only patients with mild to moderate Alzheimer's disease were included in the above-mentioned trials, because of the early suggestion that patients with more severe disease do not benefit from tacrine therapy; (iii) patients with dementing disorders other than Alzheimer's disease would not be expected to benefit from the therapy with acetylcholinesterase inhibitors since the mechanisms of these disorders do not primarily involve the loss of cholinergic neurons (however, it has been recently proposed (Liberini et al., 1996) that patients with Lewy-body dementia (another common cause of dementia with even more severe cholinergic damage than Alzheimer's disease) might be more responsive to acetylcholinesterase inhibitors); (iv) so far, there have been no data on the safety of acetylcholinesterase inhibitors in Alzheimer's disease patients with psychiatric problems (e.g., depression) or behavioural problems (aggressive behaviour), or with various systemic diseases, which, nevertheless, are frequent in elderly patients and require additional therapies.

4. Possible reasons for the limited efficacy of the inhibitors of acetylcholinesterase in Alzheimer's disease

While it is hoped that new trials on available drugs or more effective new drugs will improve the situation of Alzheimer's disease therapy, it is time for a critical reappraisal of the problem. The following are some conceivable reasons for the present failure.

4.1. The genetic, neurochemical, neuropathological, and clinical heterogeneity of the disease and patients

The genetic, neurochemical, neuropathological and clinical heterogeneity of the disease and patients (Cutler and Sramek, 1997; Eagger and Harvey, 1995) and the differences in pharmacokinetics and the conceivable hindrance to inducing selective pharmacological response in neuroanatomical target areas are some of the reasons for the present failure. The observations that only subgroups of patients respond to acetylcholinesterase inhibitors implies that the diagnosis covers a cholinergically heterogeneous group. Unfortunately, so far, there is no reliable biological or clinical marker to identify potential responders, though various potentially predictive measures have been proposed (blood levels of the drugs, positron emission tomography, EEG, Apolipoprotein E).

In order to claim that a treatment has delayed the progression of the disease by, for instance, 1 year, it is necessary to conduct a parallel study comparing the drug and placebo for at least 1 year. The course of Alzheimer's disease is so variable and unpredictable that the course of the decline cannot be extrapolated from the initial slope of change (Growdon, 1993).

4.2. Adverse effects can make it necessary to reduce the doses

The tolerability of drugs intended for use in old, debilitated patients is obviously critical. A high incidence of adverse effects decreases the quality of life and may jeopardize compliance. A careful balance of efficacy and tolerability is therefore necessary to justify imposing an additional strain on body systems that may already be damaged by the normal ageing process (Wagstaff and McTavish, 1994). These adverse effects of acetylcholin-

esterase inhibitors may be either peculiar to a drug or group of drugs (e.g., hepatotoxicity for tacrine), or intrinsic to this class of compounds, e.g., the peripheral cholinergic effects. On the whole, the side-effects limit the number of patients who can tolerate higher, more effective, doses.

4.3. The phasic properties of cortical acetylcholine function

The prevalently phasic properties of cortical acetylcholine function make it difficult for increased acetylcholine in the synaptic cleft to eventually result in stimulation of post-synaptic receptors independently from pre-synaptic activity. Excessive autoreceptor stimulation may eventually reduce the ability of pre-synaptic neurons to transmit properly (Sarter and Bruno, 1997; Sarter et al., 1997).

4.4. The alterations of cholinesterases in the Alzheimer's disease brain

Earlier studies had shown that overall acetylcholinesterase activity is decreased in Alzheimer's disease cortex and hippocampus, making the use of acetylcholinesterase inhibitors questionable. More recent observations have confirmed these data, but have also shown the complexity of the problem.

First, the relative proportion of membrane-bound G4 form (by far the prevalent and physiologically critical form of acetylcholinesterase in the cholinergic neurons of the central nervous system, CNS), was selectively decreased in various Alzheimer's disease brain areas, whereas the much less abundant G1 was unchanged. This resulted in a 40–80% lower G4/G1 ratio which correlated with the loss of choline acetyltransferase activity and with the histopathology (Arendt et al., 1992; Fernandez et al., 1996; Ogane et al., 1992a; Schegg et al., 1992; Siek et al., 1990; Younkin et al., 1986).

Second, while acetylcholinesterase activity was markedly reduced in the remaining normal cells and axons of the Alzheimer's disease cortex, histo-immunocytochemical studies revealed the presence, from the early stages of the disease, of new, enzymatically atypical cholinesterases associated with the amyloid in senile plaques and cerebral microvessels and with the paired helical filaments in neurofibrillar tangles. These new forms differed from the acetylcholinesterase in normal neurons and axons as to pH requirement, lower substrate inhibition and sensitivity to conventional inhibitors (reviewed by Geula and Mesulam, 1995). The relationship between acetylcholinesterase and amyloid was further substantiated by the finding of an increased expression of acetylcholinesterase in cell cultures exposed to β-amyloid (Sberna et al., 1997).

Third, in contrast with acetylcholinesterase, the activity of butyrylcholinesterase (acylcholine acylhydrolase, EC 3.1.1.8), which was low in the normal mammalian brain, was increased in the Alzheimer's disease brain, especially in the glia of temporal and enthorinal cortex, two regions highly susceptible to Alzheimer's disease pathology. The butyrylcholinesterase was also co-localized with senile plaques (Younkin et al., 1986), concomitantly with progressive β-amyloid aggregation, suggesting that it has a role in the maturation of the plaques (Gómez-Ramos and Morán, 1997).

Thus, there is growing evidence that cholinesterases, probably through their 'non-cholinergic' functions, could participate in the pathological processes leading to the formation and/or deposition of β-amyloid (reviews by Appleyard, 1992; Small et al., 1996. See also Inestrosa et al., 1996; Wright et al., 1993). The presence of different forms of cholinesterases in Alzheimer's disease makes it difficult to identify the pharmacological target and might significantly contribute to the wide variability of the response to known inhibitors.

The pharmacology of acetylcholinesterase molecular forms has not been extensively investigated, and the results of the studies were somewhat divergent. In vitro, experimental studies on rat brain soluble forms of acetylcholinesterase indicated that ENA 713 and eptastigmine are stronger inhibitors of G1 than of G4 (Enz et al., 1993). Conversely, physostigmine, tacrine and metrifonate inhibited both G1 and G4 forms (either soluble or membrane-bound) with similar potency (Ogane et al., 1992a).

The relative in vitro specificity of eptastigmine for G1 compared to that for G4 and the lack of specificity of physostigmine were similar in the control and Alzheimer's disease brains (Ogane et al., 1992b). Another study showed that physostigmine and tacrine were much more effective to inhibit acetylcholinesterase of normal axons and pericarya from control brains than either the acetylcholinesterase or the butyrylcholinesterase from senile plaques and neurofibrillar tangles (Wright et al., 1993). Moreover, tacrine, physostigmine and donepezil were inactive on the fraction isolated from senile plaques, but inhibited the solubilized fraction from the control and Alzheimer's disease brains and the G4 from the rat brain (Mimori et al., 1997). Thus, some drugs seem to inhibit the physiological, rather than the pathological, form of acetylcholinesterase. Whether this activity is beneficial or detrimental is still unknown.

4.5. Alterations of receptor response in Alzheimer's disease

A critical assumption of the cholinomimetic therapy in Alzheimer's disease (either with acetylcholinesterase inhibitors or acetylcholine receptor agonists) is that the post-synaptic muscarinic receptors are expressed in measurable amounts despite the neurodegeneration, and that signal transduction remains intact. Muscarinic receptors

represent the majority of acetylcholine receptors in the brain and most of them (M_1, M_3) are coupled to phosphoinositide hydrolysis through the $G_{q/11}$ protein.

Despite some conflicting reports, there is an almost general consensus that muscarinic M_1 receptors are spared in Alzheimer's disease (reviews by Giacobini, 1990; Greenamyre and Maragos, 1993; Nordberg, 1992; see also Ohara et al., 1994). However, studies on the level of mRNA gave inconclusive results (Harrison et al., 1991; Wang et al., 1992).

Of crucial importance is the evidence that M_1 signal transduction is disrupted in Alzheimer's disease (reviews by Cowburn et al., 1996; Flynn et al., 1995; Horsburgh and Saitoh, 1994; Jin and Saitoh, 1995; Jope, 1996; Ladner et al., 1995. See also Crews et al., 1994; Garlind et al., 1995; Greenwood et al., 1995; Haug et al., 1996; Jope et al., 1997; Wang and Friedman, 1994; Wang et al., 1994; Warpman et al., 1993). This impairment occurs at a number of sites: (i) receptor-G protein coupling, in terms of high-affinity binding, receptor-stimulated phospholipase C, GTPγS binding and GTPase activity; (ii) cholinergic activation of phosphoinositide hydrolysis with acetylcholine plus acetylcholinesterase inhibitors (tacrine, physostigmine, neostigmine), or with various muscarinic receptor agonists (40–50% lower than controls); (iii) [^{3}H]inositol 1,4,5-triphosphate receptor; and (iv) protein kinase C activity and translocation. The alteration of the (βII) form appeared to be particularly relevant since this form was associated with diffuse plaques (Masliah et al., 1991), which are considered an early marker of Alzheimer's disease pathology.

Interestingly, the activity of phosphatidylinositol 4-kinase in cortical membranes was reduced in Alzheimer's disease patients and that in control subjects was inhibited in vitro by β-amyloid, suggesting other potential sites of phosphoinositide alteration in Alzheimer's disease (Wallace, 1994). It should be noted that changes in protein kinase C in Alzheimer's disease were not confined to the brain, but were also present in fibroblasts (Govoni et al., 1993), confirming the hypothesis that there are also peripheral alterations in Alzheimer's disease.

There were only few exceptions to the reported alterations of phosphoinositide hydrolysis. The more relevant exception (from the Bowen's group) attributed the above-mentioned results to inadequate consideration of the effects of terminal coma and post-mortem autolysis (Alder et al., 1995). This problem has been adequately discussed (Greenwood et al., 1995; Jope, 1996).

The dysfunction of cholinergic signalling in Alzheimer's disease which was described for various cortical regions of these patients and correlated with the relative abundance of neuritic plaques, may result in altered modulation of the proteolytic processing of the APP, thus of amyloidogenesis. In turn, experimental studies have recently shown that β-amyloid peptides at very low concentrations (picomolar to nanomolar) alter cholinergic neurotransmission at various sites, including the cholinergic signalling (Auld et al., 1998).

Very briefly, the APP is an integral membrane protein that can be processed either: (i) by a secretory mechanism (α-secretase) giving rise to soluble, non-amyloidogenic fragments (APPs) in the extracellular space, or (ii) by amyloidogenic pathways (β-secretase) producing β-amyloid peptides (39–42/43 amino acids) that can aggregate and eventually form amyloid plaques (review by Yankner, 1996).

The complex mechanisms of APP processing appear to operate simultaneously in cells, but their relative activities are modulated by G protein-coupled neurotransmitter receptors that increase α-secretase and inhibit β-secretase processing (reviews by Nitsch, 1996; Nitsch and Growdon, 1994).

In vitro, muscarinic receptor agonists, such as carbachol, decreased the level of β-amyloid and enhanced the secretion of APPs in transfected as well as non-transfected cells (Farber et al., 1995; Nitsch and Growdon, 1994; Wolf et al., 1995). This effect appeared to be specific for muscarinic M_1/M_3 receptors and occurs through activation of protein kinase C.

These in vitro findings suggested that muscarinic receptor agonists and acetylcholinesterase inhibitors could reduce β-amyloid formation, therefore affecting the course of the disease (Giacobini, 1996; Nitsch, 1996). So far, the impairment of signal transduction does not support such a suggestion. Only further extensive investigations (both experimental and clinical) will prove whether this hypothesis is valid.

As regards the acetylcholinesterase inhibitors, this caution is even more justified, as the experimental studies on their possible effects on APP processing yielded controversial results. The administration of eptastigmine (but not of physostigmine or metrifonate) decreased the levels of APP-Kunitz-type protease inhibitor mRNA in the rat cortex (Giacobini et al., 1995). In vitro, elevated APPs release (not associated with change in the level of total APP mRNA) was reported for rat cortical slices perfused with physostigmine, eptastigmine or 2,2-dichlorovinyldimethyl phosphate (a metabolite of metrifonate) (Giacobini et al., 1995; Mori et al., 1995b). Conversely, the secretion of APPs in a number of cell lines was strongly inhibited by treating cells with tacrine, which only marginally reduced the level of intracellular APP (Lahiri et al., 1994).

4.6. The cholinergic system is not the only neurotransmitter system affected in Alzheimer's disease

Changes in glutamatergic systems were suggested by the reduced binding and uptake of D-[^{3}H]aspartate and the loss of other putative markers (Palmer and Gershon, 1990). The secretion of somatostatin and other neuropeptides was also altered (review by Valenti, 1996). Marked losses were reported in the serotoninergic neurons of the raphe nuclei

(in connection with a decrease in cortical serotonin and 5-hydroxyindolacetic acid), and in the noradrenergic neurons of the locus ceruleus in association with a decrease in cortical levels of noradrenaline and dopamine-β-hydroxylase (Palmer, 1996). Monoaminergic alterations were less severe and widespread and occurred later than those of the cortical cholinergic innervation. Nevertheless, such alterations might account for the behavioural symptomatology of Alzheimer's disease, and contribute to the failure of the cognitive cholinergic therapy.

There have been only few studies on the effect of acetylcholinesterase inhibitors on monoamines. The rat cortical levels of noradrenaline and dopamine (but not of serotonin) were markedly enhanced by s.c. donepezil, and, to a lesser extent, by eptastigmine and metrifonate (Giacobini, 1996; Giacobini et al., 1996; Mori et al., 1995a). In vitro, tacrine inhibited monoamineoxidase A and increased the release of catecholamines and serotonin from rat brain slices. Conversely, in vivo tacrine had either no effect or decreased the monoamines in the rodent brain (Wagstaff and McTavish, 1994), but raised the concentration of homovanillic acid and 5-hydroxyindolacetic acid in the cerebrospinal fluid of patients (Alhainen et al., 1993).

Cholinergic and catecholaminergic systems are known to reciprocally modulate learning and memory (Decker and McGaugh, 1991; Santucci et al., 1991). Some experimental findings regarding the synergistic interaction between the α_2-adrenoceptor antagonists, idazoxan and yohimbine, with eptastigmine (Camacho et al., 1996; Cuadra and Giacobini, 1995) suggest that a combination of cholinergic and adrenergic drugs may represent a significant advantage in Alzheimer's disease treatment.

5. Conclusions

The concerted effort devoted to the strategy of acetylcholinesterase inhibition was prompted by the identification of one of the many possible targets in a multifactorial pathology such as Alzheimer's disease. However, to date, the objective advantages of this Alzheimer's disease therapy are limited and there is nothing to suggest that the situation will change soon.

There may be a number of reasons for the modest efficacy of these compounds. The reasons are related to the following factors: (i) the heterogeneity of the disease and patients; (ii) the drugs, especially as regards their pharmacokinetics and side-effects; and (iii) the targets: the complex nature of acetylcholine system, the acetylcholinesterase alterations in Alzheimer's disease, the reduced response of receptors, the involvement of other neurotransmitter systems.

Indeed, the treatment with acetylcholinesterase inhibitors results, at best, in a symptomatic improvement, but does not affect the course of the disease, as also shown by the temporary nature of their action.

The relative contribution of these factors is unknown. However, given the fundamental role they could play, they should be taken into due account in the evaluation of the possible strategies and the design of new drugs for Alzheimer's disease therapy.

A final comment concerns the fact that, although the aforementioned factors have been known for sometime, the strategy of acetylcholinesterase inhibition continues to be intensively pursued. One could wonder whether alternative strategies aimed at truly tackling the course of the disease might not be worth following (Aisen and Davis, 1997; Williams and Arneric, 1997). This does not necessarily imply that acetylcholinesterase inhibitors are useless. It is tempting to speculate that a more effective approach might combine a 'disease-modifying' strategy (e.g., modulation of protein processing, the anti-β-amyloid, the anti-inflammatory, or the antioxidant) with the cognitive strategy.

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