REVIEW

Modulation of Brain Acetylcholine Levels with Cholinesterase Inhibitors as a Treatment of Alzheimer Disease

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Abstract

The author's hypothesis is that adequate acetylcholine (ACh) hydrolysis inhibition acting through a physiological mechanism could maintain levels of the neurotransmitter sufficient to stimulate postsynaptic receptors which are still active in the brain of patients affected by senile dementia of Alzheimer type (SDAT). We postulate that the use of reversible and specific cholinesterase inhibitors (ChEI) at optimal concentrations and with a suitable route of administration would be more effective and less toxic than direct cholinergic stimulation by means of cholinomimetic agents. In order to formulate new strategies of treatment with ChEI we should take into consideration distinct differences of these drugs in their effects upon ACh levels in brain. In this review we will describe experimental approaches in animals and experimental treatment in humans which may help us to find optimal levels of ChE inhibition and ACh increases in brain. The effect of repeated doses of ChEI on serum and red blood cell (RBC) ChE activity are also discussed and correlated to the effect of acute and chronic administration on brain ACh. Severity of CNS symptoms seem to correlate more closely to percent of ACh increase in brain than to percent ChE inhibition at peak time or to time of recovery of ChE activity. These experimental data on animals indicate that "slow release" drugs or intracerebral administration of ChEI may be more effective in raising ACh levels in brain and, therefore, more suitable for therapy of Alzheimer patients.

Key words: Alzheimer, cholinesterase inhibitors, brain acetylcholine
Introduction

Cholinergic function is selectively and irreversibly affected in senile dementia of Alzheimer type (SDAT). Reduction of cholineacetyltransferase (ChAT) activity in the cortex of Alzheimer patients correlates with the degree of cognitive impairment as well as with the severity of the neuropathological terminal changes of Alzheimer disease. Thus, a direct relationship between loss of forebrain cholinergic cells and cortical cholinergic innervation, biochemical damage and symptoms of SDAT seems likely (Fig. 1). In addition, transient memory enhancement with the cholinesterase (ChE) inhibitor physostigmine (Phy) administered orally, s.c. or i.v. has been demonstrated in Alzheimer patients (for review c.f. Giacobini, 1987). The mechanism invoked to explain such an improvement is the decreased hydrolysis of acetylcholine (ACh) following acetylcholinesterase (AChE) inhibition by Phy and the consequent increase in ACh levels in central cholinergic synapses, particularly in those that are related to memory circuits. We postulated that an adequate ACh hydrolysis inhibition acting through a physiological mechanism could maintain levels of neurotransmitter sufficient to stimulate postsynaptic receptors and that such mechanism would be more effective and less toxic than direct cholinergic stimulation by means of cholinomimetic agents. In order to formulate new strategies and effective modes of administration of cholinesterase inhibitors (ChEI) we should take into consideration the marked differences of these drugs in their effects on brain ACh. In this review we will discuss both new and classic experiments in animals and treatment in humans with ChE inhibitors which may help us to achieve maximal therapeutical effects at non-toxic levels of ChE inhibition and ACh increase in brain.

Fig. 1 Relationship among pathological and biochemical findings in the cortex and clinical findings in senile dementia of Alzheimer type.
Effects of acute and chronic administration of cholinesterase inhibitors on brain acetylcholine. Correlation with CNS symptoms

Acute administration of ChEI to rodents increases ACh levels in various brain areas to a peak concentration which varies from 18 to 105% depending on the type of agent, the dosage and the region (Table 1). Peak ACh accumulation after single dosage, is generally reached within a period of 5–45 minutes depending on the route of administration. This peak is generally posterior to the maximum of ChE inhibition.5,6 The increase in ACh levels reflects a presynaptic, as well as a synaptic cleft accumulation presumably depending on ChE inhibition (Fig. 2). This accumulation may influence negatively the synthesis of ACh (Fig. 2). The rate of ACh synthesis in brain increases when ACh is released, however, transmitter levels are constant at rest suggesting that a feedback mechanism is operative in controlling ACh synthesis.7 This control mechanism could depend on the concentration of ACh present within the terminal itself or near the site of synthesis (Fig. 2). Another possible mechanism could be an inhibitory effect of the high concentration of ACh on ChAT activity (Fig. 2). Acetylcholine may as a competitive inhibitor toward choline (Ch), the inhibitory constant Ki being in the range 27–100 mM.8–10 The activity of ChAT in rat brain is lowered by 45% in the presence of 100 mM ACh.9 It has been calculated that the intracellular ACh concentration at equilibrium equals to 171 μM.9 However, this does not include vesicular ACh and ACh close to ChE sites.7 Thus, even a 100% increase in ACh concentration, due to the effect of AChE inhibition (as reported in Table 1) could hardly become inhibitory to ChAT activity and to ACh synthesis. In addition, a direct inhibitory effect of acetylcholinesterase inhibitors (AChEI) on ChAT activity should be considered11 though this was not found to be true in in vitro experiments12,13 or in developing chick brain.17

Several AChEI have been tested chronically in order to produce ACh increases. The administration of a constant low dose (0.3 mg/kg i.p.) of paraxoxon to rats for 7 days does not produce symptoms until three days of treatment.12 Acetylcholine levels increase 50% during the first three days and remain fairly constant thereafter while ChE activity declines steadily during the four days of treatment to less than 20% of control activity. These results suggest that CNS symptoms are correlated with an increase in total brain ACh and that a rise of 50% above control or higher, may trigger the appearance of more severe symptoms such as tremor and convulsions. A second point is that in acutely treated animals, total ACh levels may increase as much as 100% whereas in chronically treated animals total ACh values increased maximally by 50%.12 This suggests a change in the mechanisms regulating ACh synthesis or release. It is possible that the high ACh levels resulting from long-term ChE inhibition may compete for the re-uptake of Ch (Fig. 2). It is generally accepted that a high percentage (70%)
Table 1  Changes of Acetylcholine and Choline Levels in Rodent Brain Following Acute Cholinesterase Inhibition

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg</th>
<th>Route</th>
<th>ACH Control Level (nmol/g)</th>
<th>% ChE Inhib.</th>
<th>% ACh Increase</th>
<th>ACh Peak Accum. Time (min)</th>
<th>% Ch Change</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physostigmine</td>
<td>0.650</td>
<td>i.m.</td>
<td>32</td>
<td>60</td>
<td>18-82</td>
<td>30</td>
<td>0</td>
<td>Hallak &amp; Giacobini, 1986 (5)</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>0.650</td>
<td>i.m.</td>
<td>30</td>
<td>82</td>
<td>48</td>
<td>30</td>
<td>—</td>
<td>Harris et al., 1978 (26)</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>0.600</td>
<td>i.p.</td>
<td>18</td>
<td>—</td>
<td>38</td>
<td>30</td>
<td>+39</td>
<td>Trabucchi et al., 1975 (27)</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>0.030</td>
<td>i.v.</td>
<td>100</td>
<td>98</td>
<td>34-320</td>
<td>20-120</td>
<td>—</td>
<td>London and Coyle, 1978 (28)</td>
</tr>
<tr>
<td>Soman</td>
<td>0.400</td>
<td>s.c.</td>
<td>3-70</td>
<td>98</td>
<td>49-52</td>
<td>15</td>
<td>-26; +18</td>
<td>Harris et al., 1978 (26)</td>
</tr>
<tr>
<td>Soman</td>
<td>0.230</td>
<td>s.c.</td>
<td>14-73</td>
<td>89-92</td>
<td>48</td>
<td>15</td>
<td>0</td>
<td>Shih, 1982 (29)</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>0.750</td>
<td>s.c.</td>
<td>19</td>
<td>82</td>
<td>105</td>
<td>15</td>
<td>—</td>
<td>Wecker et al., 1977a (12)</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>0.230</td>
<td>s.c.</td>
<td>14-73</td>
<td>98</td>
<td>49-52</td>
<td>15</td>
<td>+26; -18</td>
<td>Wecker &amp; Dettbarn, 1979 (15)</td>
</tr>
<tr>
<td>Metrifonate</td>
<td>125</td>
<td>i.p.</td>
<td>15</td>
<td>80</td>
<td>60</td>
<td>45</td>
<td>—</td>
<td>Nordgren et al., 1978 (30)</td>
</tr>
<tr>
<td>Metrifonate</td>
<td>80</td>
<td>i.m.</td>
<td>37</td>
<td>70</td>
<td>25-60</td>
<td>45</td>
<td>0</td>
<td>Hallak &amp; Giacobini, 1978 (6)</td>
</tr>
<tr>
<td>DFP</td>
<td>1</td>
<td>s.c.</td>
<td>221-345</td>
<td>—</td>
<td>50-70</td>
<td>120</td>
<td>0</td>
<td>Potter et al., 1985 (31)</td>
</tr>
<tr>
<td>DFP</td>
<td>1</td>
<td>i.m.</td>
<td>25</td>
<td>76</td>
<td>14</td>
<td>24 hr</td>
<td>-16</td>
<td>Russell et al., 1981 (23)</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>50</td>
<td>p.o.</td>
<td>21-55</td>
<td>87-91</td>
<td>48-71</td>
<td>15</td>
<td>+14; +59</td>
<td>Modak et al., 1975 (32)</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>15</td>
<td>i.v.</td>
<td>34-66</td>
<td>80-92</td>
<td>30-32</td>
<td>5</td>
<td>—</td>
<td>Stavinoha et al., 1976 (33)</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>0.075</td>
<td>i.m.</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>—</td>
<td>Harris et al., 1978 (26)</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>5</td>
<td>i.p.</td>
<td>18</td>
<td>83</td>
<td>30</td>
<td>30</td>
<td>+84</td>
<td>Trabucchi et al., 1975 (27)</td>
</tr>
<tr>
<td>Atropineb</td>
<td>15-16</td>
<td>i.m.;</td>
<td>14, 25</td>
<td>30</td>
<td>0</td>
<td>20; -32</td>
<td>30; +15; +45</td>
<td>Harris et al., 1978 (26)</td>
</tr>
<tr>
<td>Atropine</td>
<td>15-16</td>
<td>i.p.</td>
<td>14, 25</td>
<td>30</td>
<td>0</td>
<td>20; -32</td>
<td>30; +15; +45</td>
<td>Wecker et al., 1977 (34)</td>
</tr>
</tbody>
</table>

a) All results are from investigations using radiometric, gaschromatographic or GCMS methods for assay of ACh levels and microwave radiation for sacrifice.

b) medulla—cerebral cortex
c) striatum
d) caudate putamen—cerebral cortex
e) hippocampus—striatum
f) cerebral cortex—medulla, peak accumulation at 15 min
g) midbrain—striatum, ChE inhibition at 15 min
h) striatum—cerebral cortex
i) Results from oxotremorine (µmol/kg) and atropine experiments are reported for comparison, atropine results relate to total, cortex and hippocampus
j) [2H0]ACh, rat whole brain, GCMS
k) cerebellum—striatum
l) brainstem—cerebral cortex
m) pmol/mg prot—striatum—frontal cortex
Fig. 2 Effect of acetylcholinesterase inhibition on the cholinergic nerve terminal in CNS. Presynaptic as well as postsynaptic effects are depicted. The increase in acetylcholine levels reflect a presynaptic as well as a synaptic cleft accumulation depending on cholinesterase inhibition. This accumulation may inhibit acetylcholine synthesis as well as acetylcholine release. In addition, acetylcholinesterase inhibition might influence acetylcholine uptake as well as inhibit cholineacetyltransferase activity directly or indirectly (through acetylcholine).
Table 2 Comparison of Acute Toxicity, Effects on Brain ACh, ACh Release, ChAT and ChE Activity and Severity of CNS Effects of Three Reversible ChE Inhibitors in the Rat*

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Maximal ChE inhibition following i.m. administration</th>
<th>Percent ACh increase in CNS</th>
<th>Inhibitory effect on ACh release (striatum)</th>
<th>Severity of CNS Symptoms**</th>
<th>Inhibition of ChAc Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phy (650 µg/kg i.m.)</td>
<td>LD₅₀ (mg/kg) 1,300</td>
<td>Peak time 5</td>
<td>% inhibition at peak time 80</td>
<td>100% recovery time (min) 120</td>
<td>48</td>
</tr>
<tr>
<td>MTF (80 mg/kg i.m.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THA (15 mg/kg i.m.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Modified from Hallak and Giacobini.13
** Severity of symptoms
  (+) = slight fasciculations and tremor.
  + = general fasciculations, salivation, tremor, some splay of hind limbs.
  ++ = fasciculations, salivation, severe tremor, splay of hind limbs, general CNS depression, convulsions.

Phy = phosstigmine; MTF = metrifonate; THA = tetrahydroaminoacridine; ChE = cholinesterase; ChAT = cholineacetyltransferase; ACh = acetylcholine.

of the Ch utilized for ACh synthesis is generated from ACh hydrolysis. Thus, a reduced availability of Ch due to lower ACh hydrolysis may contribute to reduce synthesis of ACh in chronically treated animals (Fig. 2). Pretreatment with Ch prior to the administration of a ChEI prevents Ch depletion in the striatum and potentiates ACh increase.12,15 However, this effect is not seen in hippocampus or cerebral cortex. Thus, regulatory mechanisms controlling synthesis of ACh appear to differ in various brain regions depending on the neuronal composition of a particular region (e.g. striatum vs. hippocampus). In addition, our recent studies show that different ChEI [Phy, metrifonate (MTF), tetrahydroaminoacridine (THA)] show markedly different effects on ACh release13 (Table 2). Severity of CNS symptoms seem to correlate closely to the percent of ACh increase in brain than to percent ChE inhibition at peak time or time of recovery of ChE activity (Table 2). Acute toxicity in animals is also different for different inhibitors (Table 2).

The effect of repeated doses of cholinesterase inhibitors on serum and red blood cell cholinesterase activity. Experimental and clinical studies

In order to initiate an effective therapy based on ChE inhibition it is crucial to
understand central as well as peripheral effects of ChEI including biochemical as well as behavioral changes.

The effect of prolonged ChE inhibition has been extensively investigated both in animals and humans. In order to determine whether repeated doses of Phy resulted in increased tolerance, Koster\textsuperscript{16} injected various doses i.v. (0.25–2 mg/kg) at various intervals (45 min–100 hrs) repeated 2–4 times. In such experiments on 34 cats there was no clear indication of a change in the sensitivity to Phy with regard to survival rate, severity of symptoms and rate of recovery from toxicity. Serum ChE activity was 20–33\% inhibited after 0.1 mg/kg and 46\% inhibited after 2 mg/kg at 30 minutes. Following DFP (0.1 mg/kg i.v.) serum ChE was 76–86\% inhibited at 20 min, 71–84\% at 4–6 hrs and was still 70\% inhibited at 24 hrs. Other studies in animals relate more closely to various effects on the brain's cholinergic systems.\textsuperscript{5}

The most comprehensive studies of long lasting treatment with ChEI in humans are those performed with DFP in myasthenic patients,\textsuperscript{17} with sarin in normal volunteers\textsuperscript{18} and with several agents in both.\textsuperscript{19} The daily i.m. administration of 0.5–2.3 mg DFP caused a rapid and sustained fall in plasma ChE activity to between 5\% and 20\% of control activity and a slower progressive decline of RBC ChE. Following cessation of the administration of DFP, plasma and RBC ChE activity begin to increase within a few hours. Because the effect of the inhibitor is irreversible, restoration of ChE activity reflects the regeneration of new enzyme protein. Those patients who received larger doses of DFP (higher than 1.5 mg daily) developed gastrointestinal symptoms, followed by CNS symptoms. The percent inhibition of plasma ChE was not directly related to the appearance or severity of the symptoms, however, RBC inhibition of ChE was related to the appearance of symptoms. Similar conclusions were reached in the study of Comroe et al.\textsuperscript{20}

It is interesting to note that when DFP was administered for longer periods at smaller dosages (0.5–1 mg/day for 50 days) symptoms were transient or absent in spite of the fact that RBC ChE activity was still 75\% inhibited. It seems, therefore, that appearance and intensity of symptoms relate better to the activity level of ChE in the CNS or PNS rather than to peripheral ChE inhibition as measured by plasma and RBC levels of activity. These results in humans support our data in rats showing a close correlation between the activity of ChE in plasma and RBC and the curve of activity of ChE in brain after intramuscular injection of MTF and Phy suggesting that inhibition of ChE in plasma may be related to changes of this enzyme in brain.\textsuperscript{6} The duration of symptoms in the CNS may also be related to the concentration of the inhibitor in that particular region. Our results in the rat\textsuperscript{5} demonstrated that following ChE inhibition by Phy, changes in levels of ACh can vary greatly from region to region (from 15\% in medulla oblongata to 80\% in cortex). We found in the rat a correlation between concentration of Phy and ChE inhibition (Fig. 3) in the CNS.\textsuperscript{6} This relation-
Conclusion

A high degree of AChE inhibition with modestly elevated (10–15%) ACh levels can be maintained by using repeated doses of both reversible and irreversible ChEI. Since the risk for neuronal damage and the frequency and severity of toxic effects is higher with the irreversible agents it seems reasonable to consider for treatment purposes only reversible agents. However, various types of reversible AChEI (Phy, THA, MTF), show significant differences with regard to their effect on brain ACh, ACh release, toxicity and duration of action\(^\text{13}\) (Table 2). Thus, control studies in animals should be helpful in suggesting the suitable agent, dosage and route of administration.\(^\text{6,13,21}\) Development of behavioral tolerance to ChEI does not seem to correlate to changes in

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Fig. 3  Correlation between concentrations of physostigmine and cholinesterase inhibitors in brain. The relationship is independent of both dosage and route of administration (Mod. from Hallak and Giacobini\(^\text{13}\)). □ = 650 μg/kg i.m.; ● = 500 μg/kg i.m.; ▲ = 100 μg/kg i.v.
concentrations of ACh, Ch, high affinity Ch transport and rate of synthesis of ACh induced by AChE inhibition either total regional or synaptosomal.22,23 Consequently, other alternatives should be considered as an explanation to tolerance such as decrease in cholinergic (muscarinic or nicotinic) receptors or release of ACh known to occur simultaneously with the development of behavioral tolerance and in relation to chronic treatment with AChEIs.24,25 Based on our experience on animals, "slow release" agents such as MTF or intracerebral administration of "fast-acting" agents such as Phy should be more suitable for a long-lasting ChE inhibition and effective ACh increases in brain of Alzheimer patients.4 Of the three ChEI tested (Phy, THA, MTF), MTF seems to be the most promising as far as long-lasting ChE inhibition effect on ACh levels in brain, and lack of toxicity at therapeutical doses. Double blind clinical trials of these three drugs presently in progress at our Alzheimer Center will show whether our prediction is accurate or not.

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