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# ALZHEIMER'S AND PARKINSON'S DISEASES

## Strategies for Research and Development


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# PHARMACOLOGICAL ACTIVITY OF NOVEL ANTICHOLINESTERASE AGENTS OF POTENTIAL USE IN THE TREATMENT OF ALZHEIMER'S DISEASE

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## INTRODUCTION

In dementia of the Alzheimer type there is a selective loss in the cerebral cortex of choline acetyltransferase (CAT), the enzyme that synthesizes acetylcholine (ACh)[1,2]. The degree of dementia and memory impairment that occurs in this condition is well correlated with the decrement in cortical cholinergic transmission[3]. Moreover, scopolamine, a cholinergic antagonist, can cause memory impairment in normal individuals similar to that in aging[4]. These findings suggest that impaired cortical cholinergic transmission may be at least in part responsible for the symptomatology of Alzheimer disease. In support of this suggestion it was found that physostigmine, which prevents the destruction of ACh, can cause memory improvement in Alzheimer patients[5]. The extent of improvement of the symptomatology was closely related to the degree of inhibition of acetylcholinesterase (AChE) in the spinal fluid, and thus to the amount of physostigmine reaching the central nervous system[6].

As potential therapy for dementia, physostigmine has a number of disadvantages, the most serious of which is its low therapeutic ratio. In most studies in which any improvement in symptomatology was reported, the dose range in which this occurred was very narrow (1-2.5mg orally[6] or 0.25-0.5mg, i.v.[7]), with higher doses causing a decrement in performance or distressing side effects due to peripheral cholinergic overactivity. Another disadvantage is its low chemical stability[8] and short duration of action, which necessitate frequent dosing. Its oral bioavailability is also unpredictable, and it only appears to produce improvement in Alzheimer symptomatology by this route if it is given with lecithin[9].

The purpose of the present study was to synthesize anticholinesterase agents which readily reach the CNS after parenteral and oral administration; which have a higher therapeutic ratio than that of physostigmine, greater chemical stability, and a longer duration of action. These advantages should make them more suitable than physostigmine for the long term treatment of conditions associated with a deficit in cholinergic transmission in the central nervous system.

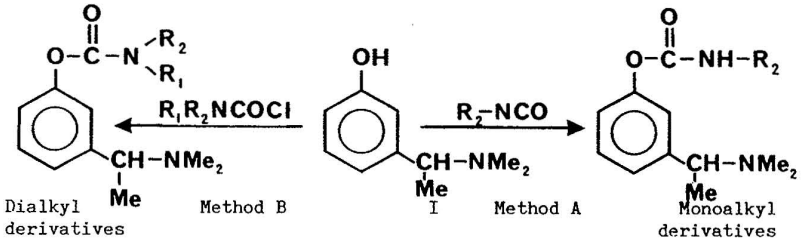
Apart from physostigmine, all of the carbamate anticholinesterases which are used medicinally, have a quaternary N-function and thus do not

penetrate the CNS to any significant extent[10]. Almost all the synthetic carbamates with a tertiary N were designed as insecticides, and have a monomethyl substituent on the N of the carbamate. They are thus relatively unstable at physiological pH and of short duration[10]. One such carbamate, miotine, has only been used clinically as a miotic[11]. The dimethyl analogue, has only been used as an insecticide[12]. The effect of other mono or dialkyl substitution on the N of the carbamate of this structure on AChE activity in vitro or in vivo does not appear to have been studied. Accordingly we prepared and tested a series of mono and alkyl derivatives of miotine, the activities of some of which are described. (A patent has been applied for the novel structures). Particular emphasis is placed on their abilities to inhibit brain AChE and on their relative toxicities.

## METHODS

### Preparation of mono- and di-substituted phenyl carbamates

The N-monoalkyl and N,N-dialkyl substituted phenyl carbamates were synthesized from α-m-hydroxyphenylethyl-dimethylamine (I), which was itself prepared according to the procedure described by Stedman and Stedman[11] with minor modifications, as shown in the scheme below:




For the synthesis of the monoalkylphenyl carbamates, a 2-3 fold molar excess of the alkyl isocyanate was reacted with phenol I in dry benzene at room temperature overnight (see Scheme 1 method A). For the synthesis of the N,N-dialkyl-substituted phenyl carbamates, 1.5-2 fold molar excess of the corresponding carbamoyl chloride was allowed to react with phenol I in dry acetonitrile in the presence of a similar excess of sodium hydride (see Scheme 1 method B). The weak acidity of phenol I required the use of a strong base such as sodium hydride to produce the phenolate which acts as the nucleophile.

All carbamates were obtained as hydrochloride salts by saturating their etheral solutions with HCl(g). These salts were purified by recrystallization from ethanol-ether. Purity was assessed by t.l.c. on precoated silica gel plates, reversed-phase HPLC, elemental microchemical analysis and $^1$H-n.m.r.

### Measurement of antiAChE activity in vitro

Male mice (Sabra strain) weighing 30-40g were sacrificed by cervical dislocation and the whole brain minus cerebellum rapidly removed and weighed. The brains from 10 mice were homogenized in 1ml/100g wet weight phosphate buffer 0.1M pH 8.0, centrifuged at 12,000 rpm and the supernatant, discarded. The pellet was mixed with a similar volume as above of buffer 0.1M pH 8.0 containing 1% Triton using a Vortex Genie at maximum speed for 1 min. The mixture was centrifuged and the supernatant which contained most of the solubilized AChE was used for subsequent determinations of anticholinesterase activity.

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