Studies on 4-Phenylpiperidine Series

X. Some Geometric Isomers of Quaternary Piperidinium Salts with Anticholinesterase Activity

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Received April 23, 1969

SUMMARY

Some isomeric 1-Me/4-Ph-*cis*- and *trans*-4-phenyl-4-formyl-*N*-methyl-*N*-alkyl (or aralkyl)-piperidinium salts have been synthesized and tested for their inhibitory activity toward acetylcholinesterase and serum cholinesterase. The enzyme activity was determined by the method of Ellman *et al.* [*Biochem. Pharmacol.* **7**, 88 (1961)]. The values for inhibitor constants (K_i) are reported. In the case of serum cholinesterase, the K_i values for *cis* isomers are generally lower than those for the corresponding *trans* isomers. In the case of acetylcholinesterase, the K_i values for the *trans* isomers are lower than those for the corresponding *cis* series. The results suggest that conformations favoring *e*-phenyl and *a*-formyl groups at C-4 will better fit the anionic site of acetylcholinesterase if the substituents at the quaternary nitrogen atom are *e*-methyl and *a*-alkyl. On the other hand, compounds can attain a closer fit to the anionic site of cholinesterase if the substituents at the quaternary nitrogen atom are *a*-methyl and *e*-alkyl groups (the positions of substituents at C-4 being unchanged).

INTRODUCTION

Conformational isomerism in the acetylcholine molecule may have an important role in determining the interaction with different receptors, such as the nicotinic, the muscarinic, and the acetylcholinesterase receptor surfaces (1). That acetylcholine interacts with nicotinic receptors in a fully staggered conformation, and with muscarinic receptors with a quasi-ring conformation, has been suggested by Archer and coworkers (2). Less information is available concerning the active form of acetylcholine in its interaction with the specific esteratic enzymes. Evidence that acetylcholine must adopt a specific conformation, when bound to the esteratic surface, comes from the fact that acetylcholinesterase hydrolyzes only one optical form of β-methylacetylcholine (3) and displays marked stereospecificity toward some series of inhibitors (4).

In the present work we have tested some 1-Me/4-Ph-*cis*- and *trans*-4-phenyl-4-formyl-*N*-disubstituted piperidinium salts as inhibitors of two cholinesterases, acetylcholinesterase and serum cholinesterase, in order to obtain information on the steric requirements of the active sites of the two enzymes.

Previous work (5) has dealt with the relative configuration and probable conformation of these isomeric pairs of compounds (Fig. 1). The *cis* isomers exist almost exclusively in the Ia conformation, with the two larger groups equatorial to the piperidine ring. In the *trans* isomers (II, R = *n*-Pr and *n*-Bu), conformer IIa is more favored than conformer IIb. For the *trans* isomer (II, R = *i*-Pr), conformation IIa is still favored, but the contribution of IIb is higher than that for the former isomers. In the case of the *trans* isomer (II, R = CH_2Ph), both conformers IIa and IIb contribute almost equally in the mixture at equilibrium.

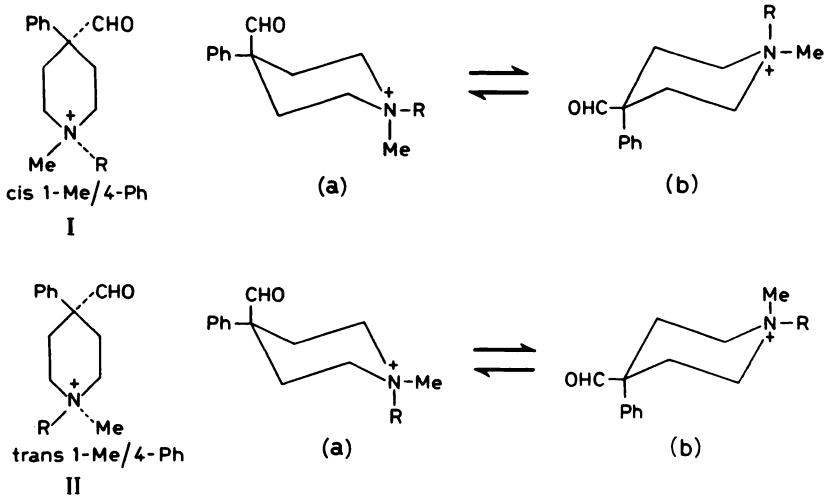

FIG. 1. *Conformations of 1-Me/4-Ph-cis- and trans-4-phenyl-4-formyl-N-methyl-N-alkyl (or aralkyl)-piperidinium salts*

MATERIALS AND METHODS

Acetylcholinesterase from *Electrophorus electricus,* purified by chromatography and gel filtration (Worthington Biochemical Corporation, Freehold, N. J.), was used for experiments with true acetylcholinesterase. Cholinesterase, a lyophilized powder from horse serum, obtained from the same firm, was used for the experiments with serum cholinesterase.

The activity of the enzymes was measured according to the procedure of Ellman *et al.* (6) with a Beckman DK-2 spectrophotometer. The final concentrations of the reagents were as follows: acetylthiocholine iodide, 5.6×10^{-3} M; phosphate buffer, pH 7.2, 3.9×10^{-2} M; 5,5′-dithiobis(2-nitrobenzoic acid), 2.1×10^{-4} M. All reagents (obtained from Boehringer, Mannheim, Germany) and the piperidinium salts were dissolved in glass-distilled water. The reaction was initiated by adding the enzyme preparation to a cuvette containing all the reagents. The values of optical density at 412 mμ were measured from the second to the sixth minute of the reaction. The nonenzymatic hydrolysis of ATCh[1] was found to be negligible. The mean values of percentage inhibition were plotted on semilogarithmic paper as a function of inhibitor concentration, and 50% inhibition values (I_{50}) were determined from curves with at least six points.

The K_m values of ATCh-AChE and ATCh-ChE complexes were determined graphically by plotting $1/v$ with respect to $1/S$ according to Lineweaver and Burk (7).

The K_i values were calculated from the relationship

$$K_i = I_{50} \frac{K_m}{K_m + S}$$

derived from the Michaelis-Menten equation for a competitive system (8).

RESULTS AND DISCUSSION

In Table 1 are reported the apparent dissociation constants (K_i) of the enzyme-inhibitor complex for 1-Me/4-Ph-*cis*- and *trans*-piperidinium salts. The competitive nature of the inhibition was verified by plotting I against $1/v$ at two different substrate concentrations (9) for two pairs of isomers, namely I and II (R = *i*-Pr) and I and II (R = *n*-Bu), chosen as examples for both series. K_m for the hydrolysis of ATCh by AChE was 1.93×10^{-4} M, and by ChE, 3.12×10^{-4} M. These values are in agreement with those reported in the literature (e.g., ref. 6).

[1] The abbreviations used are: ATCh, acetylthiocholine iodide; AChE, acetylcholinesterase; ChE, cholinesterase.

The K_i values of *cis* isomers for ChE were generally lower than those for the corresponding *trans* isomers, while the K_i values of *trans* isomers for AChE were lower than those for the corresponding *cis* series. Table 1 shows that in the interaction of *cis* isomers with ChE, the K_i values remained constant with chain length from C_1 to C_4, whereas a regular increase of K_i can be observed in the case of interaction of *cis* isomers with the AChE system. A similar relationship, although less marked, can be observed for the *trans* isomers.

substitution in the equatorial methyl group increases binding for the system I (R = *i*-Pr)–ChE over that for the interaction of I (R = *i*-Pr) with AChE.

From these results and from the ratio of K_i values reported in Table 1, it is evident that the *trans* series displays more affinity than the *cis* series for AChE, and the reverse is true for ChE (an exception is made for the isomeric pair of benzyl derivatives, which have about the same inhibitory activity toward the two enzymes). Lengthening of the alkyl straight chain in the *cis* series,

TABLE 1

Inhibition of cholinesterase and acetylcholinesterase by 1-Me/4-Ph-cis- and trans-4-phenyl-4-formyl-N-methyl-N-alkyl (or aralkyl)-piperidinium iodides

R	Isomer	Cholinesterase		Acetylcholinesterase	
		K_i	$K_i(cis)/K_i(trans)$	K_i	$K_i(cis)/K_i(trans)$
		M		M	
Me		6.58×10^{-5}		3.86×10^{-6}	
n-Pr	*cis*	6.00×10^{-5}		2.73×10^{-5}	
n-Pr	*trans*	2.77×10^{-4}	0.22	8.65×10^{-6}	3.15
n-Bu	*cis*	5.90×10^{-5}		7.65×10^{-5}	
n-Bu	*trans*	2.21×10^{-4}	0.27	4.16×10^{-5}	1.83
i-Pr	*cis*	6.85×10^{-6}		1.29×10^{-6}	
i-Pr	*trans*	2.74×10^{-5}	0.25	5.66×10^{-7}	2.27
CH_2Ph	*cis*	5.58×10^{-6}		1.16×10^{-5}	
CH_2Ph[a]	*trans*	3.16×10^{-6}	1.76	9.32×10^{-6}	1.24
Eserine[b]		7.11×10^{-7}		4.99×10^{-7}	

[a] As the chloride.

[b] The change in absorbance from the first to the second minute was used to calculate the velocity.

The benzyl derivatives showed a decrease in affinity for AChE as compared with that of the *N*-dimethyl derivative, and an increase in the affinity for ChE, which displayed more adaptability to the changes in the alkyl side chain. In the latter case the benzyl group can improve binding to the enzyme through attraction forces due to the aromatic ring (10).

The substitution of 2 hydrogen atoms in the axial methyl group of I and II (R = Me) for two methyl groups improves the adsorption of the compound to the enzymatic surface, and this effect is more pronounced in the interaction of II (R = *i*-Pr) with AChE. On the other hand, the same

in fact, does not influence the affinity toward ChE, but decreases the affinity toward AChE. This regular decrease in the series which displays less adaptability to the enzymatic surface of AChE is consistent with increasing destabilization of the enzymatic complex as a result of enhanced interference in the approach of the ring to the protein surface. The $\Delta(\Delta F)$ value has been found to be —0.45 kcal/mole at 25° for any additional methylene group (11, 12).

As IIa is the favored conformation for the 1-Me/4-Ph-*trans* isomers (R = *n*-Pr, *n*-Bu, and *i*-Pr), we believe than an axial alkyl substituent, rather than an equatorial one, can account for a better fit to the

anionic site of AChE. The reverse is true for ChE: here *cis* isomers are more active than *trans* isomers. As the 1-Me/4-Ph-*cis* isomers exist almost exclusively as Ia conformers, we suggest that an equatorial alkyl substituent accounts for a closer fit with the anionic site of ChE.

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