SUBSTITUTED PYRIDINIUM DERIVATIVES AS INHIBITORS OF ACETYLCHOLINESTERASE

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Summary

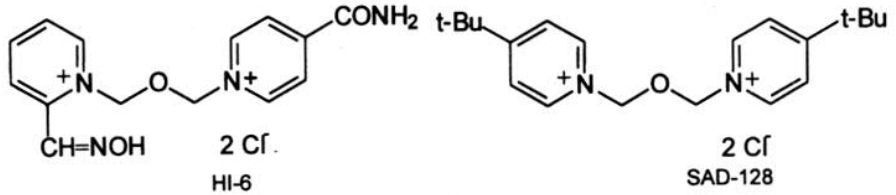
The interaction of twenty-six different 2-, 3-, and 4- substituted N-methylpyridinium iodides with acetylcholinesterase (AChE) was investigated in vitro experiments. All compounds involved in this study were found to be competitive AChE inhibitors with K_{diss} values ranging from 5.5 to 130 μM.

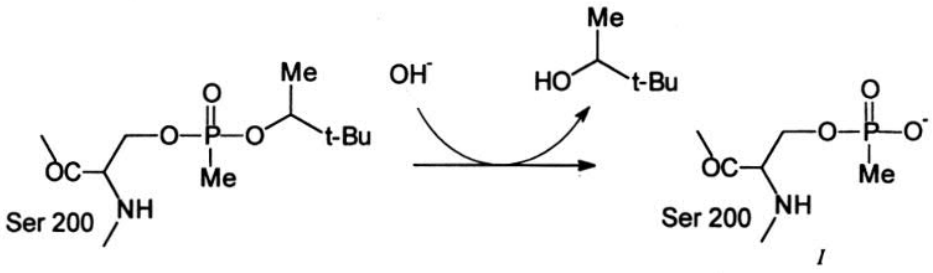
KEY WORDS: Acetylcholinesterase; Inhibition; Pyridinium salts.

Introduction

Acetylcholinesterase (AChE) has a considerable capacity for binding structurally diverse ligands, and there is a substantial evidence that there exist several binding sites in this enzyme (1-3). One of them is the anionic subsite which is an integral part of the catalytic centre of AChE (4). There also exists another, so-called peripheral anionic centre (5). Anionic centres are important binding sites for different ligands possessing positive charge in their molecule (6). The anionic subsite binds the charged quaternary group of the choline moiety of acetylcholine, and also other quaternary ligands (7), and quaternary oximes, which often serve as effective reactivators of organophosphate-inhibited AChE. Quaternary heteroarenium salts (e.g. pyridinium) are known to be good ligands of AChE (6, 8). These compounds exhibit very often strong pharmacological effect (9). Among them, AChE inhibitors (10, 11), compounds protecting AChE against inhibition effect of organophosphates (12) or carbamates (13, 14), and, in particular, reactivators of AChE inhibited by organophosphates (15-18) can be found. In this study, twenty six N-methyl-pyridinium iodides possessing different types of substituents in 2-, 3- or 4-positions (Table I) were investigated *in vitro* as reversible inhibitors of rat brain AChE.

The research was inspired by the fact that some bisquaternary pyridinium salts with different types of substituents (e.g. aminocarbonyl and *tert*-butyl) as HI-6 and SAD-128 reduce substantially the rate of pinacolin alcohol release from AChE inhibited by Soman (19).

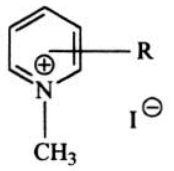




Scheme 1

Table I

Chemical structures of substituted pyridinium iodides and K_{diss} values for their interaction with acetylcholinesterase



R	K_{diss} (M)		
position of substituent	2	3	4
$CONH_2$	$1.27 \cdot 10^{-3}$	$5.42 \cdot 10^{-4}$	$4.94 \cdot 10^{-4}$
$CONH\text{-}CH_3$		$5.42 \cdot 10^{-4}$	$4.95 \cdot 10^{-4}$
$CONH\text{-}CH_2CH_3$	-	$6.30 \cdot 10^{-4}$	$6.67 \cdot 10^{-4}$
$CONH\text{-}NH_2$		$3.93 \cdot 10^{-4}$	$7.84 \cdot 10^{-4}$
$COCH_3$	$1.50 \cdot 10^{-4}$	$3.05 \cdot 10^{-5}$	$4.75 \cdot 10^{-5}$
CN	$1.40 \cdot 10^{-5}$	$1.06 \cdot 10^{-4}$	$4.03 \cdot 10^{-5}$
CH_3	$2.26 \cdot 10^{-5}$	$6.00 \cdot 10^{-5}$	$5.45 \cdot 10^{-5}$
CH=O	$3.80 \cdot 10^{-4}$	$1.10 \cdot 10^{-4}$	$2.60 \cdot 10^{-4}$
CH=NOH	$2.10 \cdot 10^{-4}$	$8.60 \cdot 10^{-5}$	$1.40 \cdot 10^{-4}$
$CH(CH_3)_3$			$2.80 \cdot 10^{-5}$
$N(CH_3)_2$			$5.50 \cdot 10^{-6}$

As a result of this process known as "aging" of phosphonylated AChE (20) (Scheme 1), phosphonate salt I of AChE is formed. Due to the strongly decreased nucleophilicity of the phosphorus atom, salt I cannot be reactivated by known reactivators as 2-PAM, TMB-4, toxogonine etc. We wondered whether there exists some relation between the type and position of the pyridinium salt substituent, its affinity towards AChE and its ability to suppress the aging of the phosphonylated enzyme.

Experiments

The quaternary pyridinium salts employed in this study were prepared by quaternization of the corresponding substituted pyridines with methyl iodide except of the two hydrazides which were obtained by hydrazinolysis of methoiodides of pyridinecarboxylic acids esters with hydrazine hydrate. All compounds were identified by their melting points, ^{1}H-NMR spectra and by elemental analyses, eventually. The syntheses were performed at the Department of Organic Chemistry, Institute of Chemical Technology, Prague, except of N-methyl-2-, 3-, and 4-pyridinium aldoximes which were prepared at the Department of Toxicology, Military Medical Academy, Hradec Králové. All other chemicals used were reagent grade and were obtained from commercial sources.

The rat brain homogenate in distilled water (10 %, w/v) was used as AChE source in all experiments. The activity of AChE was determined by pH static titration of acetic acid released from acetylcholine iodide at pH 8.0 and 25 °C. Alkaline hydrolysis of acetylcholine iodide at the same conditions was taken as a blank in all cases. The titrations were performed on an automatic Radiometer titrator RTS 822 (Copenhagen) as follows: in distilled water (18 ml) and 3 M NaCl (2.5 ml) in the titration vessel, rat brain homogenate (0.5 ml) together with aqueous solution of tested compound of known concentration (2.0 ml) and 0.020 M acetylcholine iodide (2.0 ml) were added at 25 °C. Acetic acid was titrated with 0.01 M NaOH. The slope of the NaOH consumption *vs* time plot was the activity of the enzyme (in fact, this slope represented the initial rate v_0 of the enzyme reaction). Thus, activity A_0 of the intact and activity A_{inh} of the inhibited enzyme were obtained.

The dissociation constants (K_{diss}) of enzyme-inhibitor reversible complex were calculated from the equation (21) *(1)*

$$A_{inh} = A_0 \cdot C_S / [C_S + K_M (1 + C_I / K_{diss})] \quad (1)$$

where C_S is molar concentration of the substrate used (1.6 mM in our experiments), C_I is molar concentration of the inhibitor, and K_M is the Michaelis constant for acetylcholine as a substrate (0.19 mM for the rat brain AChE).

Results and Discussion

All the tested pyridinium salts behave as reversible inhibitors of AChE. The dissociation constants K_{diss} representing their affinity to the enzyme, were calculated from equation (1) for several different concentrations of the inhibitor (5-8 values) in each experiment. Representative plots illustrating the dependence of AChE inhibition (in per cents) on molar concentration of several selected pyridinium inhibitors are given in Figs. 1-3. In all cases, sigmoidal dose-response curves were obtained.

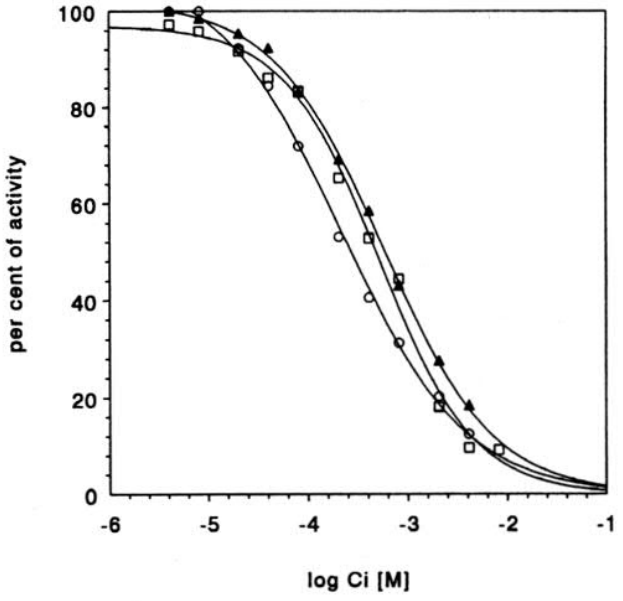


Fig. 1 The inhibition of rat brain acetylcholinesterase by three isomers of methyl-pyridinium-methiodides in vitro. The dependence of per cent of inhibition on molar concentration of 2- (○), 3- (▲), and 4-methylpyridinium-methiodide (□), eventually.

The dissociation constants K_{diss} of all tested pyridinium inhibitors and their chemical structures are given in the Table I. All the tested compounds were weak or medium reversible AChE inhibitors as apparent from the obtained values of K_{diss}, ranging from 5.5 μM (4-dimethylamino-1-methyl-pyridinium iodide) to 1.27 mM (2-carbamoyl-1--methylpyridinium iodide). As expected, the K_{diss} values are influenced predominantly by the type of substituent. The influence of the substituent position is less significant. The K_{diss} values obtained in our experiments are in accord with the results of the previous studies in the series of halogenated pyridinium salts (6) (K_{diss} ranging from 8 to 34 μM),

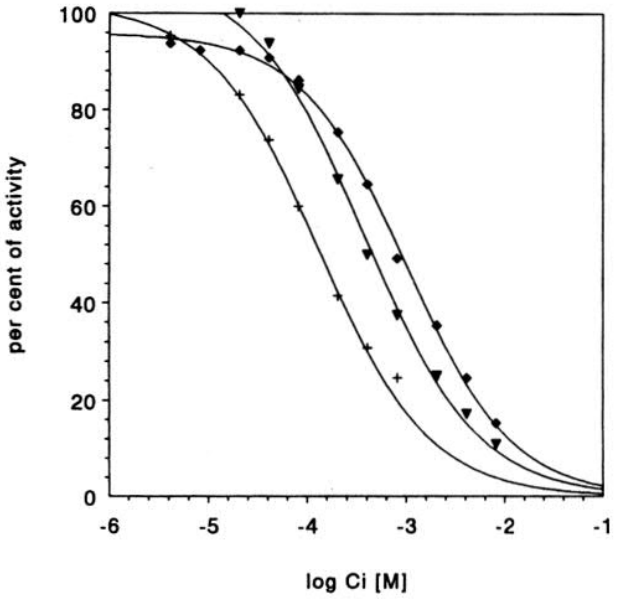


Fig. 2 The inhibition of rat brain acetylcholinesterase by three isomers of cyano-pyridinium-methiodides in vitro. *The dependence of per cent of inhibition on molar concentration of 2-(✚), 3-(◆), and 4-cyano-pyridinium-methiodide (▼), eventually.*

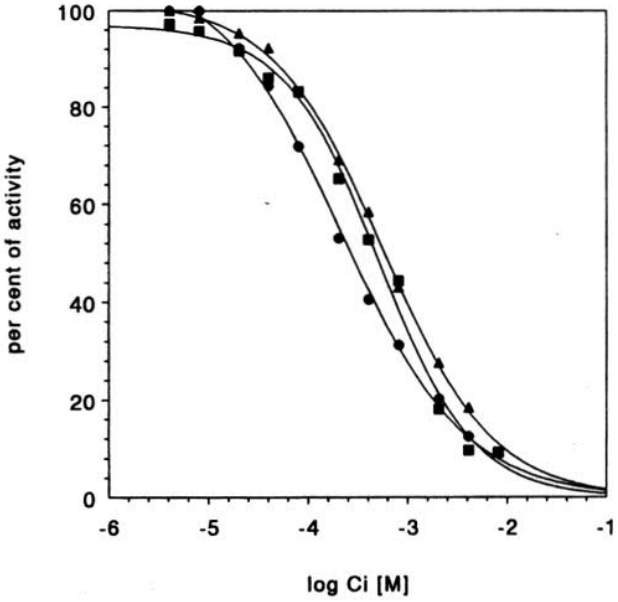


Fig. 3 The inhibition of rat brain acetylcholinesterase by three isomers of acetyl-pyridinium-methiodides in vitro. *The dependence of per cent of inhibition on molar concentration of 2-(●), 3-(▲), and 4-acetyl-pyridinium-methiodide (■), eventually.*

N-methyl-alkylpyridinium salts (9) (K_{diss} ranging from 5 to 17 μM) and N-methylpyridiniumaldoximes (18, 22, 23).

The obtained results give no evidence of the relationship between the type of the pyridinium salt substituent, its affinity towards AChE and its ability to suppress the aging of the phosphonylated enzyme - although both *tert*-butyl and carbamoyl group in bisquaternary pyridinium salts suppress

the aging of phosphonylated AChE, their affinities towards the enzyme differ in more than one order of magnitude.

Acknowledgements

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