is not clear, but it might be probably explained by the difference in the methods used for the measurement of oximes in the blood.[10] The species differences might be of some importance as well.[11]

The intramuscular administration of oximes gives a rapid absorption from the site of injection but in this case, too, the blood level of TMB-4 was somewhat higher during the 3-hr period than that of Toxogonin. At the end of the experimental period the mean concentration of Toxogonin was about 1–2 μg/ml while the concentration of TMB-4 was twice this amount. If the concentration of 0·1–0·2 μg/ml of Toxogonin is regarded adequate for a therapeutic effect in organophosphorus compounds poisoning [3] the blood concentration of 1–2 μg/ml of Toxogonin must be considered as a very effective one. Since there is no substantial difference in reactivating potencies between TMB-4 and Toxogonin,[5, 12] it can be expected, with a high degree of probability, that the therapeutic effectiveness of this oxime will be at least equal if not greater than that of Toxogonin.

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Choline acetyltransferase inhibitors: a group of styryl-pyridine analogs

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A GROUP of styryl-pyridine analogs that inhibit choline acetyltransferase have been studied. The most potent of these was hexamethylene-1-4-(1-naphthylvinyl)-pyridinium-6-trimethylammonium dibromide with an I_{50} value of 9×10^{-7} M. The anti-choline acetylase activity appears to be associated with the large conjugated, planar, lipophilic moiety. The trimethylammoniumalkane appendage does not seem to be a qualitatively critical structure and contributes less to choline acetylase inhibitory activity than to cholinesterase inhibition. The most specific inhibitor for choline acetylase is 4-(1-naphthylvinyl)-pyridine with an I_{50} of 3×10^{-5} M, which inhibits cholinesterase only at high concentrations.

Choline acetyltransferase (choline acetylase; acetyl-CoA: choline O-acetyltransferase, EC 2.3.1.6; ChA) is responsible for the synthesis of acetylcholine (ACh) in nervous and other tissues. A variety of types of compounds have been reported to be weak inhibitors of this enzyme (reviewed by Nachmansohn[1]). Few of these have I_{50} values below 10^{-3} M. The availability of a potent and specific ChA inhibitor would be of great interest in further elucidating the role of this enzyme in brain and

in other tissues. Compounds of this type are described in this report.* Some of the structural features associated with ChA inhibitory activities among this group of compounds also were investigated. A preliminary report of some of this work has been presented previously.[6]

METHODS

ChA activity was assayed by a modification of the method of McCaman and Hunt.[7] The source of ChA used in this study was a 100,000 *g* supernatant of a 1:30 aqueous homogenate of rat cerebral cortex. The inhibitors were freshly dissolved in distilled water before addition to the assay system. Preincubation of the inhibitor with the enzyme did not affect the observed inhibition.

The effect of the various compounds on the hydrolysis of ACh by human red cell cholinesterase (acetylcholine acetyl hydrolase, EC 3.1.1.7; AChE) and human plasma cholinesterase (acylcholine acyl hydrolase, EC 3.1.1.8; BuChE) was determined by a null-point potentiometric titrimetric procedure using a pH-Stat (Radiometer, Copenhagen). The final substrate concentrations employed were $3{\cdot}0 \times 10^{-3}$ M and $2{\cdot}0 \times 10^{-2}$ M for AChE and BuChE respectively. The assays were carried out at 37° with 0·1 N NaOH as titrant. The I_{50} values were obtained by drawing a smooth curve through the points obtained from the mean of triplicate analyses of at least five inhibitor concentrations.

RESULTS AND DISCUSSION

Since ChA and AChE are the enzymes which, respectively, synthesize and hydrolyze ACh, a degree of similarity between their active sites might be anticipated. Both enzymes should possess active sites capable of interacting with the quaternary ammonium group of ACh. Consequently, competitive inhibition by quaternary ammonium compounds might be anticipated. In contrast, compounds such as diisopropylphosphorofluoridate (DFP), which inhibit AChE by formation of a phosphoryl derivative of the serine residue at the region of the active site,[8] would not be expected to inhibit ChA. ChA activity also requires a sulfhydryl group and is inhibited by sulfhydryl reagents such as *p*-chloromercuribenzoate, iodoacetate, and *N*-ethyl maleimide, which have I_{50} values of about 10^{-5} M in a crude ChA system.

In preliminary studies ChA was found to be inhibited by high concentrations of choline and ACh. Phosphocholine (10^{-3} M) caused a 22 per cent inhibition. A number of other monoquaternary compounds gave similar values. Among bisquaternary ammonium compounds, 5×10^{-3} M decamethonium and hexamethonium inhibited ChA 10 per cent and 41 per cent respectively. Hexafluorenium, a hexamethonium analog in which the planar 9-fluorenyl ring replaces one methyl group on both nitrogens, has an I_{50} value of 10^{-3} M. Dequalinium chloride, which contains two quinolinium moieties separated by a decamethylene chain, has an I_{50} of 2×10^{-4} M. It seemed worthwhile to examine the anti-ChA effect of some quaternary pyridinium compounds. The most potent inhibitor encountered so far has one quaternary pyridinium function, which is part of a large, planar, conjugated lipophilic group linked by a hexamethylene chain to a trimethylammonium moiety. The I_{50} value of this compound (hexamethylene-1-4-(1-naphthylvinyl)-pyridinium 6-trimethylammonium dibromide; I) for ChA is 9×10^{-7} M. It previously has been reported for its neuromuscular blocking properties.[9]

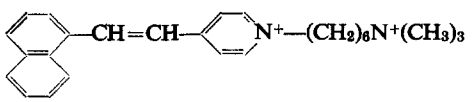
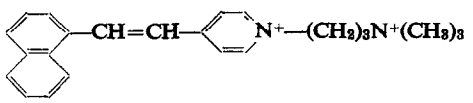
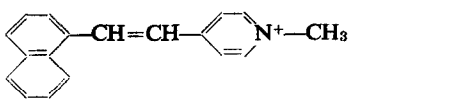
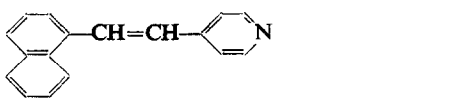
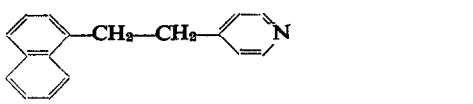
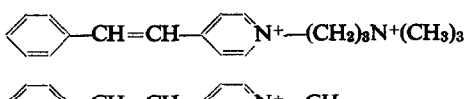
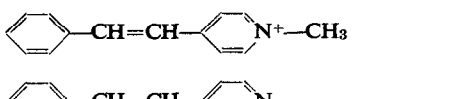
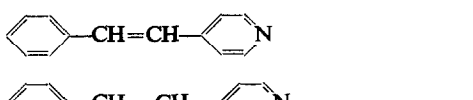
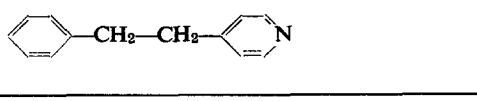
The influence of some structural modification of (I) on anti-ChA effects can be seen in Table 1. Shortening the methylene chain linking the two nitrogens from six to three (II) reduced the inhibitory potency by a factor of 30. Replacement of the naphthyl group of II with a phenyl group (VI) further reduced the I_{50} by a factor of 10. Elimination of the trimethylammoniumalkane appendage of I while retaining the quaternized feature of the pyridinium nitrogen yielded compound III, which is one-third as active as I, but ten times more potent than the *bis*-quaternary II. The tertiary analog of III (IV) shows a 10-fold reduction in its I_{50} value. The phenyl analog of IV (VIII) is one-fifth as active. Saturation of the ethylenic double bond of IV (V) and of VIII (IX) caused a profound reduction in inhibitory potency.

The strong ChA inhibitory activity of I appears to be related to the presence of a large, conjugated, coplanar, lipophilic structure with a diffuse positive charge. The trimethylammoniumalkane appendage seems to contribute relatively little to its anti-ChA activity. This appendage structure, however,

* Most of these compounds were supplied by Neisler Laboratories, Inc., Decatur, Ill., and were prepared by published methods.[2–5]

appears to contribute significantly to anti-AChE activity. In fact, I is a more potent inhibitor of AChE than of ChA. II is an equipotent inhibitor of these two enzymes; the styryl-pyridinium analog, VI, has greater anti-AChE than ChA activity. The simple metho-quaternized derivative (III) is more specific for ChA. The unquaternized pyridine compounds are poor inhibitors of AChE, and IV, which retains considerable anti-ChA activity, is the most specific inhibitor of the series for ChA. It produces more than 95 per cent inhibition of ChA at 6×10^{-4} M, a concentration at which it has no demonstrable anti-AChE effect. All the quaternary pyridinium derivatives are less potent inhibitors of BuChE than of AChE.

TABLE 1. STRUCTURAL FORMULAE OF A GROUP OF CHOLINE ACETYLTRANSFERASE INHIBITORS AND THEIR MOLAR I_{50} VALUES FOR CHOLINE ACETYLTRANSFERASE (ChA), ACETYLCHOLINESTERASE (AChE) AND BUTYRYLCHOLINESTERASE (BuChE)

		I_{50} ChA	I_{50} AChE	I_{50} BuChE
I		9×10^{-7}	6×10^{-7}	3×10^{-6}
II		3×10^{-5}	3×10^{-5}	1×10^{-4}
III		3×10^{-6}	4×10^{-5}	2×10^{-5}
IV		3×10^{-5}	4% at 10^{-3}	0% at 10^{-3}
V		6% at 10^{-3}	10% at 10^{-4}	1.6×10^{-4}
VI		4×10^{-4}	4×10^{-5}	4×10^{-5}
VII		1.5×10^{-5}	3×10^{-5}	1×10^{-4}
VIII		6×10^{-4}	0% at 10^{-3}	29% at 10^{-3}
IX		0% at 10^{-3}	10% at 10^{-3}	0% at 10^{-3}

These styryl-pyridine derivatives and their naphthyl analogs are presumed to be trans-isomers. The conjugated ethylenic linked cyclic systems favor a coplanar configuration, and this would be sterically prohibited in a *cis*-configuration of the 1-naphthyl derivative. The relatively high activity of III is interesting in that the cationic charge is spread rather than concentrated on the pyridinium nitrogen. The significant activity of IV is still more surprising because of its very weak basic character. These observations, together with the importance of the conjugated system for activity, strongly

suggest receptor bonding interactions involving van der Waals and hydrophobic bonding, and possibly charge-transfer complexing. These factors are being further investigated.

The kinetics of ChA inhibition are being studied with the most potent inhibitor, I. The most specific inhibitor of ChA, IV, is being employed for the investigation of the inhibition of ChA *in vivo* on various physiological functions. All the compounds which inhibit ChA show reversible non-competitive inhibition.

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The stoichiometry of erythromycin binding to ribosomal particles of *Staphylococcus aureus*

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ERYTHROMYCIN, a macrolide antibiotic, inhibits bacterial protein synthesis, both *in vivo*[1] and *in vitro*.[2,3] Recently, erythromycin was reported to be bound to ribosomes isolated from *Escherichia coli*[4] and *Bacillus subtilis*.[5] Some evidence suggests that the inhibition of protein synthesis is related to the binding of erythromycin to the ribosomes,[5,6] but the number of erythromycin molecules bound per ribosome was not established. Because these data on binding of erythromycin to ribosomes would appear more significant if a definite number of erythromycin molecules were bound to each ribosome, the stoichiometry of the binding was studied.

S. aureus 209P was grown in Brain Heart Infusion (Difco) and harvested at the early expoential phase of growth. Ribosomes were isolated and washed twice with standard buffer by the method for