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FURTHER STUDIES ON THE CONFORMATION OF ACETYLCHOLINE

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SUMMARY

A reexamination of the hypothesis that acetylcholine exists in a cyclic conformation was undertaken. The determination of infrared-absorption spectra and the acylation rates of a number of analogues of acetylcholine were carried out. The assembled evidence indicated that the shift toward the higher energy of the infrared absorption of the carbonyl peak of acetylcholine and its higher rate of acylation argues against a cyclic conformation and for an inductive influence of the quaternary nitrogen.

INTRODUCTION

In an earlier communication, we reported that acetylcholine exhibits unique activation of its carbonyl group[1]. This was characterized by a shift in the infrared-absorption spectra toward the higher energy, as well as by an increased rate of acylation of hydroxylamine. From these properties of acetylcholine, we proposed a cyclic conformation for acetylcholine, in solution, of an ion-dipole character brought about by an electrostatic interaction between the quaternary nitrogen and polarized carbonyl oxygen.

There remained, however, the possibility that the quaternary nitrogen may manifest its effect on the carbonyl group by an inductive influence through the choline carbon chain, and thus we launched an investigation to examine this point. We would like to report our further finding which indicates that while this proposal of a cyclic conformation still appears to be substantially correct, the acyl activation is indeed influenced by an inductive effect through the chain from the quaternary nitrogen.

MATERIALS AND METHODS

Preparation of acetyl compounds

2-Tripropylaminoethylacetate chloride: 14 g (0.1 mole) of tripropylamine and 8 g (0.1 mole) of redistilled 2-chloroethanol were heated at 95–100° in a sealed tube for 4 h. The reaction mixture was freed of unreacted tripropylamine and chloroethanol in a rotary evaporator. The residue was treated with a seven times excess of acetic anhydride and refluxed for 8 h. The crude product was recrystallized from alcohol–

ether; m.p. 153–154°. (Found: C, 58.11; H, 10.36. $C_{13}H_{28}O_2NCl$ requires C, 58.73; H, 10.62 %.)

2-Tributylaminoethyl acetate chloride: This material was prepared in a manner similar to the above; m.p. 65–67°. (Found: C, 60.56; H, 10.84. $C_{16}H_{34}O_2NCl$ requires C, 62.41; H, 10.62 %.)

Trimethylaminomethyl acetate chloride was prepared by the method of RENSHAW AND WARE[2]. Cyanomethylacetate was prepared by the method of HENRY[3]. Acetoxyethylacetate was prepared by the method of GAL[4]. β-Nitroethylacetate was prepared by the method of HENRY[5].

Infrared spectra and acylation rate studies

The infrared spectra were determined with a Perkin–Elmer 221G instrument programmed for maximum resolution. A fixed-path sodium chloride cell and a variable sodium chloride reference cell were used throughout the measurements. The esters were dissolved in absolute ethanol immediately before measurements were carried out.

The acylation velocity rates of the homologues of acetylcholine were carried out at 25° and at 0° in water. At zero time, 10 ml of 2 M hydroxylamine hydrochloride dissolved in 0.2 M glycine buffer and adjusted to pH 10.2 was added to approximately 10 mg of the ester in 10 ml of solvent. 1-ml samples were removed at intervals and immediately pipetted into 0.2 ml 4 N HCl to stop the reaction. After all of the samples were taken, 0.5 ml of 10 % $FeCl_3$ in 0.1 N hydrochloric acid followed by 2 ml of ethanol, were added to the samples to develop the hydroxamate color. The absorbancy was read in a spectrophotometer at 520 mμ.

RESULTS

The infrared-absorption spectra of the homologues of acetylcholine in the 2000 cm^{-1} to 1600 cm^{-1} region were examined and compared with the rates with which these substances acylate hydroxylamine (Table I). Only acetylcholine (2-trimethylaminoethyl acetate chloride) and acetylformocholine (trimethylaminomethyl acetate chloride) exhibit a single carbonyl absorption peak. A relationship emerges between the homologues and their energy of absorption, as well as between the homologues and their rates of acylation of hydroxylamine.

The infrared-carboxyl absorption spectra and the acylation rates for 2-tripropylaminoethyl acetate chloride and 2-tributylaminoethyl acetate chloride are given in Table II. It is noteworthy that both esters behave substantially like acetylcholine and arrange in a pattern; the larger the alkyl, the slower the acylation rates. The infrared-absorption energy of both esters is somewhat lower than of acetylcholine.

The results of a study of non-quaternary type acyl activated esters are given in Table III. It is noteworthy that the shift in the infrared-absorption spectra for carbonyl stretching frequencies as well as the acylation rates are in accord with those observed in the quaternary series.

DISCUSSION

The data assembled above forces a reexamination of the extent to which a cyclic conformation of acetylcholine in solution participates as a molecular species. The in-

creased acylation rates can be accounted for by either of two possible hypotheses. One is the cyclic conformation discussed in an earlier publication[1], and the other arises from the inductive influence of the quaternary nitrogen through the carbon chain which would alter the carbonyl stretching absorption and activate the acyl group (see Figure). Thus, for ethyl acetate (A) the unshared electron pair or the ester oxygen is free to attenuate the partial positive charge on the carbonyl carbon, while in acetylcholine (B) the inductive influence of the quaternary nitrogen restricts this effect. The question, then, is which effect is operating—the cyclic conformation, the inductive effect, or both; and if both, to what extent does each participate?

From the data in Table I, the relationship of the distance of the quaternary nitrogen from the ester group allows one to conclude that either effect might be operative, but favors an inductive effect, since as the quaternary nitrogen is moved closer to the ester oxygen, the effect becomes more pronounced. One cannot explain the properties of the acetylformocholine with the cyclic conformation alone. We undertook then to evaluate the extent to which either effect operates to influence the molecule. Our approach was based upon the assumption that the larger alkyl groups substituted for the three methyl groups on the nitrogen would sterically hinder the formation of a cyclic conformation, while the inductive effect of the quaternary nitrogen would be only slightly mitigated by these larger alkyl groups.

TABLE I

RELATIONSHIP BETWEEN ACETYLATION RATE AND CARBONYL ABSORPTION PEAK TO INCREASING CHAIN LENGTH IN THE CHOLINE HOMOLOGUE SERIES

Acetyl ester	*Relative acylation rates*		*Carbonyl absorption peak (wave numbers)*
	($K_{0°}$)	*($K_{25°}$)*	
$CH_3-C(=O)-O-CH_2-N^{(+)}(-CH_3)_3$ Trimethylaminomethyl acetate	9.0	—	1780 cm^{-1}
$CH_3-C(=O)-O-CH_2-CH_2-N^{(+)}(-CH_3)_3$ Trimethylaminoethyl acetate	0.21	1.24	1760 cm^{-1}
$CH_3-C(=O)-O-CH_2-CH_2-CH_2-N^{(+)}(-CH_3)_3$ Trimethylaminopropyl acetate	—	0.085	1752 cm^{-1} 1732 cm^{-1}
$CH_3-C(=O)-O-CH_2-CH_2-CH_2-CH_2-N^{(+)}(-CH_3)_3$ Trimethylaminobutyl acetate	—	0.019	1748 cm^{-1} 1728 cm^{-1}

One can observe from the data in Table II that there is a slight decrease in both the rates of acylation and the energy of the carbonyl absorption in the infrared with increasing size of the alkyl substituent. Thus, this data would argue that the inductive effect exercises a greater impact on the state of the molecule, but there is some evidence for the cyclic character of the molecule. The fact that increasing the size of the alkyl

substituent decreases the acylation rates and the energy of absorption can be interpreted as evidence for the cyclic conformation, or that there is appreciable mitigation of the charge on the quaternary nitrogen by the larger alkyl groups; however, the fact that this effect is small, argues for the inductive effect.

The behavior of the non-quaternary type "active" esters in Table III demonstrated that acyl activation of similar character to the quaternary esters, can take place without the possibility of a cyclic conformation, and lends credence to the importance of the inductive effect.

TABLE II

RELATIONSHIP BETWEEN ACETYLATION RATE AND CARBONYL ABSORPTION PEAK TO THE SIZE OF THE N-SUBSTITUTED ACETYLCHOLINE DERIVATIVES

Acetyl ester	*Relative acylation rates (K_{e^0})*	*Carbonyl absorption peak (wave numbers)*
$CH_3-C(=O)-O-CH_2-CH_2-N^{(+)}(-CH_3)_3$ Trimethylaminoethyl acetate	0.21	1760 cm^{-1}
$CH_3-C(=O)-O-CH_2-CH_2-N^{(+)}(-CH_2-CH_2-CH_3)_3$ Tripropylaminoethyl acetate	0.167	1754 cm^{-1}
$CH_3-C(=O)-O-CH_2-CH_2-N^{(+)}(-CH_2-CH_2-CH_2-CH_3)_3$ Tributylaminoethyl acetate	0.155	1754 cm^{-1}

TABLE III

ACETYLATION RATE AND CARBONYL ABSORPTION PEAK OF SOME NON-QUATERNARY TYPE ACTIVE ACYL ESTERS

Acetyl ester	*Relative acylation rates (K_{e^0})*	*Carbonyl absorption peak (wave number)*
$CH_3-C(=O)-O-CH_2-C\equiv N$ Cyanomethyl acetate	1.0	1761 cm^{-1}
$CH_3-C(=O)-O-CH_2-C(=O)-OCH_2-CH_3$ Carboxyethylmethyl acetate	0.4	1758 cm^{-1} 1748 cm^{-1}
$CH_3-C(=O)-O-CH_2-CH_2-N(=O)=O$ β-Nitroethyl acetate	0.1	1754 cm^{-1} 1736 cm^{-1}

In our previous communication, we demonstrated the influence of the quaternary nitrogen of acetylcholine on two parameters; the acylation rates of the ester, and the absorption of the carbonyl group in the infrared. We concluded that these observations might best be explained with the cyclic conformation. Our present data indicates we were in error, particularly our interpretation of the energy of absorption in the infrared. The shift toward the higher energy of the carbonyl infrared absorption can best be explained as evidence for a decrease in the polarization of the carbonyl group which would argue against a cyclic conformation and in favor of the inductive influence. Of course, as we have shown, both influences may operate, but clearly, the inductive influence predominates.

What emerges from this investigation is the unique nature of acetylcholine and its uniqueness resides in its "active" acyl group. As we have shown, the presence of the quaternary nitrogen activates the acyl group largely through an inductive influence. If nothing else, the "active" acyl group provides means through hydrolysis by which this powerful neurohumoral can be "turned off" rapidly by acetylcholine esterase and is perhaps one of the basic reasons for the natural selection of this molecule in neural transmission throughout much of the animal kingdom.

Whatever this simple ester does to the biological membrane to alter its character would reasonably be assumed to involve this chemical property. Thus, we reason that acetylcholine, when it unites with its appropriate receptor on the membrane, acylates the membrane to alter its permeability. The hydrolysis of the acyl group from the membrane restores the character of the membrane. This hypothesis is currently being investigated.

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