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The Preferred Conformation of the Muscarinic Agent L(+) Acetyl-β-Methylcholine

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Abstract It has been postulated that acetylcholine exhibits both nicotine and muscarinic activity because of its ability to present two patterns of essential atoms to the receptors. These two patterns arise from the ability of the molecule to exist in more than one preferred conformation. The molecule *S*(+)-acetyl-β-methylacetylcholine exhibits only muscarinic activity. Calculations using molecular orbital theory predict that this molecule prefers only the muscarinic conformation. This is offered as an explanation for the exclusive role of the molecule and as evidence supporting the twoconformation, two-activities hypothesis.

Introduction

Acetylcholine is a chemical transmitter in two types of nervous system structures in the body. The first structure, termed the muscarinic system, is located at the junction between a nerve ending and a smooth muscle. Acetylcholine, as well as muscarine, muscarone and other agents termed "muscarinic", transmit impulses from the nerve to the smooth muscle. This activity is selectively blocked by compounds such as atropine.

The second nervous system structure employing acetylcholine is located at the junction of two nerve cells midway between the spinal cord and the smooth muscle. This activity is mimiced by nicotine and phenylcholine ether and is termed "nicotinic". This activity is blocked by compounds such as curare.

The structural requirements for the two activities appear to be different. Acetylcholine is the natural mediator for both activities, however, compounds minicing muscarinic activity usually do not produce much nicotinic activity and vice versa. Likewise, compounds blocking one effect are usually uninfluential in blocking the other effect.

The observation that acetylcholine can be responsible for two distinctly different biological effects has lead to the proposal that the molecule may exist in more than one preferred conformation in solution, thereby offering to the two receptors, different patterns of critical atoms[1,2].

Theoretical calculations using molecular orbital theory on the conformation of acetylcholine have revealed that the ethanolammonium portion prefers an approximately gauche conformation[3], *Fig.* 1*a*. This prediction has been confirmed by NMR analysis[4]. The same theoretical studies predict a conformation around the ether methylene bond as shown in *Fig.* 1*b*. Finally, the calculations predict a range of conformational preference around the carbonyl to oxygen bond as shown in *Fig.* 1*c*.

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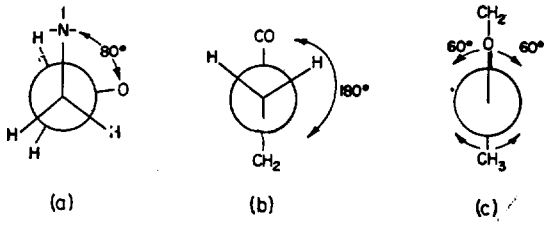

Fig. 1. Calculated conformation of acetylcholine (a) Onium and ether oxygen relationship (b) Carbonyl and methylene relationship and (c) Carbonyloxygen and methylene relationship.

The prediction of the variable conformation allowed for acetylcholine enabled Kier to propose that two patterns of essential atoms in two conformations were responsible for muscarinic and nicotinic activity[5,6], supporting earlier suggestions[1,2]. The mascarinic pattern of atoms was thus proposed to be as shown in *Fig.* 2 and the nicotinic pattern as shown in *Fig.* 3. More recent calculations have shown that the muscarinic agent oxotremorine[7] and the nicotinic agent phenylcholine ether[8] possess the appropriate hypothesized patterns of atoms.

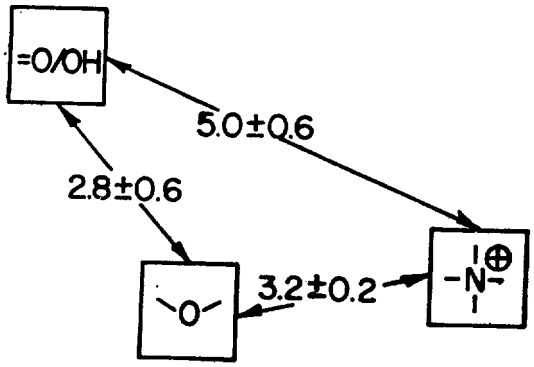

Fig. 2. Calculated pattern of a atoms predicted to be essential for muscarinic activity.

The molecule $S(+)$ acetyl-β-methylcholine, in which a methyl group is added to the carbon β to the onium group in acetylcholine is reported to be as active a muscarinic agent as acetylcholine[9]. In contrast to acetylcholine, however, the molecule is a very weak nicotinic agent. It is reasonable to suppose that the presence of the extra methyl group influences the conformation of the moleculele in such a way as to alter the nicotinic pattern and destroy that activity while retaining the muscarinic pattern. It is then of value to consider the preferred conformamation of $S(+)$-acetyl-β-methylcholine to determine if an altered conformation can account for the absence of nicotinic activity.

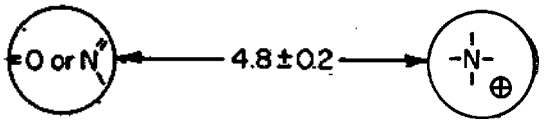

Fig. 3. Calculated pattern of a atoms predicted to be essential for nicotinic activity.

Calculation

In previous studies we have successfully employed a semi-empirical molecular orbital theory to predict molecular conformation[10,11]. The method we have used is known as extended Huckel theory developed by Hoffmann[12]. The expansion of a molecular orbital as a linear combination of atomic orbitals yields

$$\Psi_i = \sum c_{ij}\phi_j$$

upon minimizing the toatl energy, a set of Huckel equations arises

$$\sum_{j=1}^{n}[H_{ij}-ES_{ij}]C_{ij}=0 \quad (i=1, 2, \cdots\cdots n)$$

where Ψ is the molecular orbital wave function, H, the Hamiltonian operator, E, the energy, S, the overlap integral, and C the orbital coefficient. The calculations are performed for the valence electrons in s and p orbitals using Slater orbitals as a basis set. The input parameters

consist of the Slater Exponent and the Coulomb integral, approximated as the valence state ionization potential. All overlap integrals are considered. The resonance integrals are approximated by the equation

$$H_{ij}=0.5K(H_{ii}+H_{jj})S_{ij}$$

with K set at 1.75 the value preferred by Hoffmann[12]. The total electronic energy is computed to be the sum of the doubly occupied molecular orbitals,

$$E_t = 2\sum E_i$$

Since all overlap integrals and all nonbonded interactions are explicitly considered, the computed total energy E_t is sensitive to the geometry of the molecule. Thus by varying the geometry and calculating the total energy, E_t for each, the minimum energy is found, which corresponds to the preferred conformation. It is therefore necessary to include the three dimensional coordinates of each atom in the conformations selected for calculation. These are derived using a vector program which locates each atom in space in Cartesian coordinates. The bond lengths used were C−C=1.54, C−N=1.47, C−O=1.47, C=O=1.23, C−H=1.09, and N−H=1.04, all in angstroms. All bond angles were assumed to be tetrahedral with the C-CO-O bond set at 120°. The quaternary nitrogen was assumed to stagger the methylene group adjacent to it and the acetyl methyl group was assumed to have a hydrogen eclipsing the carbonyl group.

The input parameters are as follows:

Coulomb integrals

Electron	Value (eV)	Electron	Value (eV)
N 2s	−26.00	O 2p	−17.76
N 2p	−13.40	C 2s	−21.40
O 2s	−35.30	C 2p	−11.40

Slater exponents

Atom	Value	Atom	Value
H	1.000	N	1.950
C	1.625	O	2.275

Results and Discussion

Calculations on the $S(+)$ acetyl-β methyl choline molecule revealed an energy minimum for the CH_2-CH_2, O-CH_2 and CO-O bonds as shown in *Fig.* 4. In the predicted preferred conformation, the ethanolammonium segment was found to be close to a gauche conformation (90°) as was acetylcholine (80°)[11]. The O-CH_2 bond was found to be similar to acetylcholine (180°) although the wider angle (200°) separating the onium group from the carbonyl group undoubtedly is a consequence of the side chain methyl interaction with the carbonyl group. The CO-O bond was found to prefermation as shown in *Fig.* 4c in contrast to the flexibility predicted for this bond in acetylcholine[1]. This difference again may likely be due to the β methyl group.

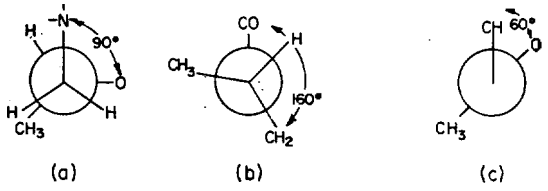

Fig. 4. Conformation predicted for $S(+)$-acetyl-β-methylcholine viewed down (a) CH_2-CH_2 bond (b) CH_2-O bond and (c) O-C bond

The crystal structure conformation of the iodide salt has been published[13]. In this form the molecule was found to have an N-CH_2-CH(CH_3)-O angle of 85°, C(O)-O-CH(CH_3)-CH_2 angle of 147° both in fairly close agreement with theoretical prediction. The O-CO-CH_2

angle in the crystal was found to be 14°, in contrast to the 60° angle calculated. A recent study using NMR and molecular rotation indicate a gauche conformarion for the ethanolammonium portion of this molecule[14].

The distance separating the carbonyl oxygen atom from the onium nitrogen atom in the predicted preferred conformation is about 4.4Å. The ether oxygen atom to onium nitrogen distance is predicted to be 3,1 Å.

If we assume the validity of these predictions, it is possible to offer an explanation as to the reason for the potent muscarininic activity of the molecule with no nicotinic activity. Muscarinic activity is apparently present due to the ability of the molecule to match the muscarinic pattern of atoms shown in *Fig.* 2. In this respect, the pattern of heteroatoms is similar to acetylcholine although the N to carbonyl oxygen distance is reduced somewhat due to the presence of the β methyl group.

The β-methyl group, however, has sufficient influence on the carbonyl oxygen atom to force it into a single preferred conformation, closer to the onium group than in acetylcholine. As a consequence, the pattern of atoms predicted for nicotinic activity in *Fig.* 3 is not met by β-methyl acethyl acetylcholin and nicotinic activity is not elicted by this molecule. This explanation and experimental evidence available are consistent with the hypothesis of dual acetylcholine activity and the muscarinic and nicotinic patterns necessary for each activity[3,5,6,11].

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References

(1) S. Archer, A. M. Lands, and T. R., Lewis, *J. Med. Pharm. Chem.*, **5**, 423 (1962).
(2) M., Martin-Smith, G. Smail, and J. Stenlake, *J. Pharm. Pharmacol.*, **19**, 649 (1967).
(3) L. B. Kier, *Mol. Pharmacol.*, **3**, 487 (1967).
(4) C. C. J. Culvenor, and N. S. Ham, *Chem. Commun.*, 537 (1966).
(5) L. B. Kier, *Mol. Pharmacol.*, **4**, 70 (1968).
(6) L. B. Kier, in "Fundamental Concepts in Drug-Receptor Interactions" (J. F. Danielli, J. F. Moran and D. J. Triggle, eds.) Academic Press, New York (1970) p. 27.
(7) L. B. Kier, *J. Pharm. Sci.*, **59**, 112 (1970).
(8) L. B. Kier, and J. M. George, *J. Med. Chem.*, **14**, 80 (1971).
(9) A. H. Beckett, N. J. Harper, J. W. Clitherow, and E. Lesser, *Nature*, **189**, 671 (1961).
(10) M. S. Jhon, U. I. Cho, L. B. Kier, and H. Eyring, *Proc. Nat. Acad. Sci.*, **69**, 121 (1972).
(11) L. B. Kier, "Molecular Orbital Theory in Drug Research", Academic Press, New York, (1971).
(12) R. Hoffmann, *J. Chem. Phys.*, **39**, 1397. (1963).
(13) C. Chothia, and P. Pauling, *Chem. Commun.*, 626 (1969).
(14) T. D. Inch, R. A. Chittenden, and C. Dean, *J. Pharm. Pharmacol.*, **22**, 954 (1970).