Structural Chemistry of Cholinergic Neural Transmission Systems. I. A Quantum Theoretical Study of the Molecular Electronic Structure of Acetylcholine

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Abstract: The results of INDO molecular orbital calculations on the molecular geometry and electronic structure of acetylcholine are reported. Local minima in the calculated potential energy surface can be identified with the crystal geometry, the geometries implicated in nicotinic and muscarinic cholinergic neural transmission systems, and the geometry favorable for hydrolysis by acetylcholinesterase. The evidence for stabilization of minima by intramolecular hydrogen bonding is considered.

The transmission of information in neural systems involves the passage of impulses of electrical current throughout a network of nerve cells (neurons). When the membrane potential of a neuron is decreased below a certain critical level, an action potential mechanism is triggered. The action potential sweeps along the axonal fiber and invades the terminal region of the cell. For impulse propagation, the excitation must then be transferred across the synapse to the next cells in sequence, be they other neurons or operator cells such as those of smooth or striated muscle. In many nervous transmission systems, this transfer is mediated by the liberation of acetylcholine, $(CH_3)_3N^+CH_2CH_2OC(=O)$-$CH_3$, which according to currently accepted theory[1] interacts with specific receptor sites; the exact sequence of events is still an area of active research.[2] This interaction effects a structural reorganization of the postsynaptic membrane, resulting in an increased ionic permeability. The influx, particularly of sodium ions, depresses the membrane potential below the critical level and initiates an action potential, thereby transmitting the nerve impulse. Acetylcholine is rapidly removed from the synaptic region by an enzyme-catalyzed hydrolysis, allowing the entire process to be repeated at a rate of several hundred times a second for brief periods.

The spatial conformation and electronic structural characteristics of acetylcholine in neurohumoral transmission are specifically complementary to some yet unelucidated structural entity incorporated in the postsynaptic membrane. A number of neurochemically active substances appear to function by adopting a geometry and electronic charge distribution resembling acetylcholine and interacting with acetylcholine receptors. Cholinergic receptors have traditionally been characterized on the basis of the biological activity of these structural analogs of acetylcholine. Data accumulated in this regard make possible a rough differentiation of acetylcholine receptors into two types: *muscarinic*, where the action of acetylcholine is mimicked by muscarine and blocked by atropine, and *nicotinic*, where the action of acetylcholine is mimicked by nicotine and inhibited by curare and hexamethonium.[3,4] Clearly an understanding of all types of cholinergic nervous transmission systems requires a detailed knowledge of the molecular electronic structural properties of acetylcholine.

From the structural formula and atomic numbering system given in Figure 1, the possible conformations of acetylcholine can be specified in terms of the dihedral angles τ (C(5)–C(4)–N–C(3)), τ(O(1)–C(5)–C(4)–N), τ (O(6)–O(1)–C(5)–C(4)), and τ(O(2)–C(6)–O(1)–C(5)). Because of the flexibility of the molecule with respect to torsional desplacements, the geometries favored under given conditions can be expected to depend somewhat on environmental effects. In view of the difficulties in taking this into quantitative account, it is valuable to have the system well characterized in the crystalline solid and *in vitro* solutions as well as in the free-space approximation generally adopted for theoretical calculations to provide a basis for studies of biological function. Progress in this area to date is reviewed in the following paragraphs.

The crystal structure of acetylcholine bromide has been investigated by Canepa, Pauling, and Sörum[5] following up earlier work by Sörum;[6] the geometry reported is shown in Figure 1. The C(5)–C(4)–N–C(3) sequence of atoms reportedly forms an antiplanar extended chain, τ(C(5)–C(4)–N–C(3)) = 180°, as expected on the basis of steric factors. The acetoxy group is planar with τ(O(2)–C(6)–O(1)–C(5)) = 0°, presumably stabilized by the partial double bond character of the C(6)–O(1) bond. Over the crystal geometries of a number of structural analogs of acetylcholine, no large variation in τ(C(5)–C(4)–N–C(3)) or τ(O(2)-C(6)-O(1)–C(5)) is observed,[4] and a specification of the geometry of acetylcholine in terms of τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)) is sufficient to characterize the three-dimensional molecular structure.

In the geometry reported for acetylcholine bromide, τ(O(1)–C(5)–C(4)–N) = 77° and τ(C(6)–O(1)–C(5)–C(4)) = 79°, henceforth denoted as {77°, 79°}. The

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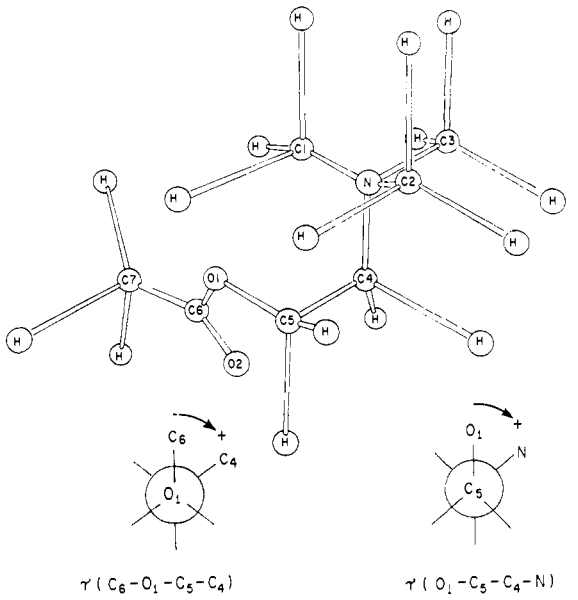

Figure 1. The structure of acetylcholine in crystalline acetylcholine bromide[5] (hydrogens not determined).

nature of the forces stabilizing τ(O(1)–C(5)–C(4)–N) in a positive synclinal conformation is of considerable interest, since an antiplanar form would be favored from purely steric considerations. The possibility of intramolecular N–C–H···O hydrogen bonding in this structure was originally suggested by Sutor[7] based on the proximity of the trimethylammonium hydrogens and ester oxygen. A number of laboratory investigations have been directed toward gaining infrared spectroscopic evidence with regard to intramolecular hydrogen bonding.[8] No strong supportive data have been reported, but the collected results are not inconsistent with such interactions. Culvenor and Ham[9] report nuclear magnetic resonance spin coupling evidence that a synclinal conformation of τ(O(1)–C(5)–C(4)–N) persists in aqueous solution as well as in the crystalline solid, but specific intramolecular hydrogen bonding was unresolvable. In a lively review, Donahue[10] discounts collected evidence for intramolecular C–H···O hydrogen bonding on the grounds that unequivocally observed C–H···O distances are not appreciably less than the expected van der Waals contact distance of 2.5–2.6 Å.

Theoretical calculations of the conformational stability of acetylcholine have been reported by Liquori, Damiani, and Elefante[11] using pairwise interaction potential functions and by Kier[12] using extended Hückel molecular orbital theory (EHT). Four energy minima in a four-dimensional potential energy hypersurface were found in the Liquori, *et al.*, analysis, two of which involve synplanar conformation of τ(O(1)–C(5)–C(4)–N) and two favoring the antiplanar conformer. The stabilizing interaction for the synplanar was attributed to an electrostatic interaction between the acyloxy oxygen and the quaternary nitrogen atom, but the analysis makes no allowance for electron exchange interactions or polarization effects undoubtedly associated with any hydrogen bonding. EHT gives also a positive synclinal τ(O(1)–C(5)–C(4)–N) of 80°.

The angle τ(C(6)–O(1)–C(5)–C(4)) specifies the orientation of the acetoxy group with respect to the rest of the molecule. This angle establishes another geometrical parameter important for biological activity: the distance between the trimethylammonium cationic head and the electronegative carbonyl oxygen atom. Early studies of acetylcholine and related compounds[13] established this distance as *ca.* 4.5 Å; this played a key role in the design of a drug (pyridine-2-aldoxime methiodide, PAM) to interact with the acetylcholine receptor on acetylcholinesterase. PAM is now used clinically to treat alkyl fluorophosphate poisoning.

In crystalline acetylcholine bromide with τ(C(6)–O(1)–C(5)–O(4)) at 79°, the acetoxy group is folded around toward the cationic head. For acetylcholine in solution, infrared spectra studies of the carbonyl vibrational frequency of acetylcholine and related compounds have been reported,[8] but the structural implications are inconclusive. The nmr spectral data[9] for acetylcholine in D_2O support an antiplanar conformation, as normally expected for a primary ester. Each of the four minima in the Liquori energy surface[11] corresponds to an antiplanar τ(C(6)–O(1)–C(5)–C(4)) as does the geometry reported for EHT calculations.[12]

Detailed consideration of the biological activities and crystal structures for structural analogs of acetylcholine implicate geometries with τ(O(1)–C(5)–C(4)–N) ranging between 60 and 120°, and τ(C(6)–O(1)–C(5)–C(4)) ranging between 144 and 213° in muscarinic action.[14] Nicotinic action has been discussed with regard to a {75°,180°} geometry by Chothia and Pauling,[15] but data summarized by Martin-Smith, Small, and Stenlake[16] point to a completely extended {180°,180°} conformer. Chothia and Pauling have also collected evidence[17] that a {150°,180°} geometry is optimal for hydrolysis by acetylcholinesterase.

With the stabilization of τ(O(1)–C(5)–C(4)–N) incompletely understood and a multiplicity of geometries implicated for the crystalline solid, solution, and various aspects of biological action, a theoretical study of the entire acetylcholine potential energy surface as a function of τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)) based on molecular quantum mechanics is in order. (The calculations reported by Kier[12] represent two slices of this surface). Our aim is to locate energy minima in the surface, to develop an understanding of the minima in terms of structural forces, and to consider the relationship between the calculated minima and molecular geometries considered in the various experimental studies described above.

The pertinent theory and methodology are reviewed in Section I, followed by Results and Discussion, and Summary and Conclusions.

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Table I. A Summary of Calculated Minima in the Acetylcholine {τ(O(1)–C(5)–C(4)–N), τ(C(6)–O(1)–C(5)–C(4))} Potential Energy Surface

Identification on Figure 2	Relative energy, kcal/mol	Geometry		Physiochemical or biological significance
		Calcd	Exptl	
A	0.00	{50°, 270°}		See text
B	3.68	{50°, 50°}	{77°, 79°}	Observed in crystalline acetylcholine bromide[a]
C	4.98	{120°, 300°}		See text
D	6.27	{40°, 180°}	{75°, 180°}	Implicated in nicotinic action[b]
			{60°–120°, 144°–213°}	Implicated in muscarinic action[c]
E	7.72	{200°, 330°}		See text
F	7.84	{160°, 30°}		See text
G	9.98	{180°, 180°}	{180°, 150°}	Implicated in nicotinic action[d] and in hydrolysis by cholinesterase[e]

[a] Reference 5. [b] Reference 15. [c] Reference 14. [d] Reference 16. [e] Reference 17.

I. Theory and Methodology

The calculation of a potential energy hypersurface for a molecule involves the calculation of the total energy of the system as a function of internal atomic displacement coordinates. In quantum mechanical systems, the energy is an expectation value of the Hamiltonian operator and molecular wave function, and thus a calculation of the wave function at a number of points in configuration space is required.

Molecular wave functions computed in this study are based on spin-restricted molecular orbital theory, with the $2n$-electron wave function Ψ considered as a Slater determinant of molecular orbitals ψ_i

$$\Psi = |\psi_1(1)\bar{\psi}_1(2)\psi_2(3)\bar{\psi}_2(4)\ldots\psi_n(2n-1)\bar{\psi}_n(2n)| \quad (1)$$

The molecular orbitals are expanded as linear combinations of atomic orbitals (LCAO) ϕ_μ centered on constituent atoms

$$\psi_i = \sum_\mu c_{\mu i}\phi_\mu \quad (2)$$

where the $c_{\mu i}$ are the linear expansion coefficients. The calculation of the molecular wave function reduces to the determination of the coefficients by matrix Hartee–Fock self-consistent field procedures.[18]

The total energy of the system at a given geometry is given by the expression

$$E = \sum_\mu\sum_\nu P_{\mu\nu}(H_{\mu\nu} + G_{\mu\nu}) + \sum_{A<B}\sum Z_A Z_B R_{AB}^{-1} \quad (3)$$

where the sums over Greek and Latin letters refer to orbitals and atoms, respectively. The first term is the electronic energy and involves $H_{\mu\nu}$, the one-electron matrix elements between atomic orbitals ϕ_μ and ϕ_ν and representative of the kinetic energy and nuclear attraction operators. The $G_{\mu\nu}$ are elements of the matrix representative of electron repulsion operators. The density matrix elements $P_{\mu\nu}$ are defined in terms of the LCAO coefficients as

$$P_{\mu\nu} = 2\sum_i^n c_{\mu i}c_{\nu i} \quad (4)$$

and specify the distribution of electronic charge in the systems. The second term in eq 3 accounts for internuclear repulsions, and involves the core charges Z_A, Z_B, and the internuclear distance between atoms A and B, R_{AB}.

The atomic and molecular integrals in this study are evaluated at the level of intermediate neglect of differential overlap (INDO).[19] The INDO method is one of a series of approximate self-consistent field molecular orbital methods developed by Pople and coworkers specifically for studies of the molecular electronic structure of large molecules. All valence electrons are treated explicitly, with the inner shell electrons and atomic nuclei considered as part of a nonpolarizable core. The INDO method has been used for conformational studies in a number of recent papers[20] and the advantages and limitations of the method are well documented.[21]

Using the INDO method, the total energy of the system is calculated by eq 3 with the appropriate integral approximations included. The net electrical charge Δq_A associated with each of the atoms A in the molecules is given by

$$\Delta q_A = Z_A - \sum_\mu^A P_{\mu\mu} \quad (5)$$

where the summation includes all $P_{\mu\mu}$ for orbitals centered on atom A.

The calculation of the acetylcholine potential energy surface as a function of τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)) involves a number of assumptions about geometrical parameters not explicity considered variable. All bond lengths reported in the acetylcholine bromide crystal structure[5] are adopted directly for our calculations and τ(C(5)–C(4)–N–C(3)) and τ(O(2)–C(6)–O(1)–C(5)) are taken as 180 and 0°, respectively. The C–H bond lengths are all taken as 1.09 Å and the methyl hydrogens of the trimethylammonium group are oriented for minimal steric repulsions.

II. Results and Discussion

The potential energy surface for acetylcholine as a function of τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)) as calculated from INDO molecular orbital theory is presented in Figure 2. Figure 2 was traced from a computer-generated contour surface based on 144 calculated grid points with contour levels spaced at intervals of 0.00035 atomic unit. The minima in the surface are labeled A, B, C...G in order of increasing energy and are summarized in Table I. Other extrema in the surface are potential energy maxima.

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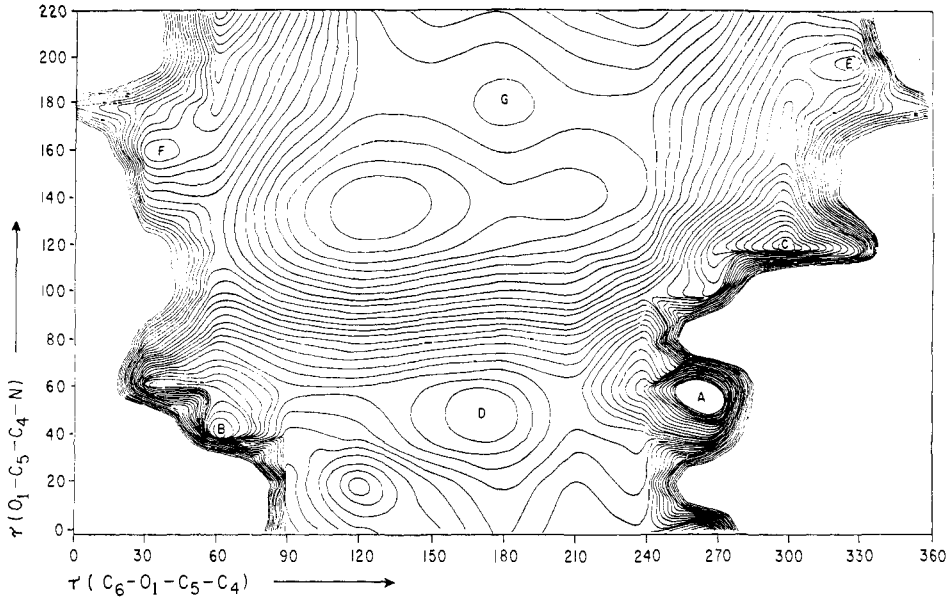

Figure 2. Calculated potential energy surface for acetylcholine as a function of τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)).

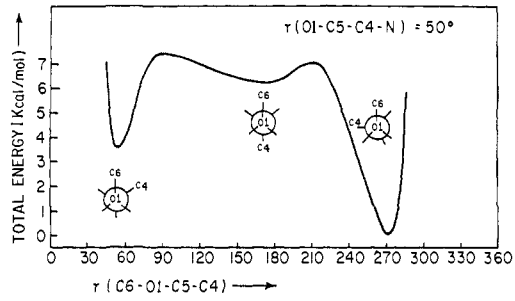

Figure 3. Calculated potential energy profile for acetylcholine as a function of τ(C(6)–O(1)–C(5)–C(4)); τ (O(1)–C(5)–C(4)–N) = 50°.

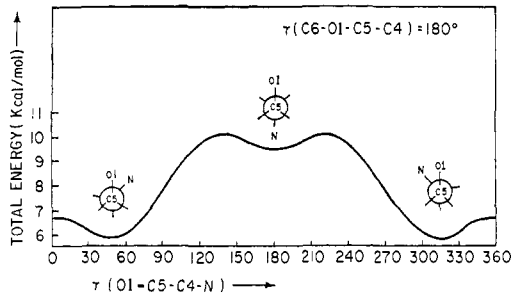

Figure 4. Calculated potential energy profile for acetylcholine as a function of τ(O(1)–C(5)–C(4)–N); τ(C(6)–O(1)–C(5)–C(4)) = 180°.

The absolute minimum on the surface, labeled A on Figure 2, is located at {50°,270°} and a local minimum at {50°,50°}, point B, is calculated to be 3.68 kcal/mol above {50°,270°}. The {50°,270°} and {50°,50°} conformers are closely related in that both positive and negative synclinal minima in τ(C(6)–O(1)–C(5)–C(4)) would be required by symmetry if τ(O(1)–C(5)–C(4)–N) were zero. With τ(O(1)–C(5)–C(4)–N) = 50°, a residual tendency toward the paired synclinal energy minima remains, showing up as points A and B on our surface.

The conformation associated with calculated minimum at {50°,50°} can be identified with the {77°,79°} geometry observed for acetylcholine in the bromide crystal.[5] While this is not the absolute minimum in our surface, a comparison of molecular models of the {50°,50°} and {50°,270°} geometries reveals the {50°, 50°} would be more favorable for closest packing of molecules in the crystalline solid.

Local minima D and G at {40°,180°} and {180°, 180°}, respectively, can each be identified with conformations relevant to biological functions of acetylcholine. The {40°,180°} conformation corresponds closely to the geometry of acetylcholine implicated in muscarinic and nicotinic aspects of cholinergic action by Chothia and Pauling.[14,15] The {180°,180°} geometry corresponds to the staggered, extended conformer considered by Chothia and Pauling[17] to be complementary to the acetylcholine receptor of acetylcholinesterase and the nicotinic conformer discussed by Martin-Smith, Smail, and Stenlake.[16]

The existence of a number of local minima calculated from quantum mechanical considerations as described above may be readily understood on the basis of fundamental principles of structural chemistry and organic stereochemistry. With τ(O(1)–C(5)–C(4)–N) held in a positive synclinal position, the calculated minima in τ(C(6)–O(1)–C(5)–C(4)) shown in Figure 3 correspond roughly to the minima of 60, 180, and 300° expected in a potential energy profile for rotation of tetrahedrally hybridized atoms about an essential single bond. With τ(C(6)–O(1)–C(5)–C(4)) held in an antiplanar position, analogous considerations apply to the minima in τ(O(1)–C(5)–C(4)–N) shown in Figure 4. The local minima at points E and F correspond to conformations wherein both τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)) fall near 60, 180, or 300°, and thus, all minima on the surface with the exception of point C can be accounted for on this basis. The local minimum at point C appears to arise from an interaction of methyl hydrogens of the trimethylammonium group and a methylene hydrogen of C(4). This interaction stabilizes τ(O(1)–C(5)–C(4)–N) at 120°. No experimental data with regard to the conformations of points A, C, E, or F have been reported.

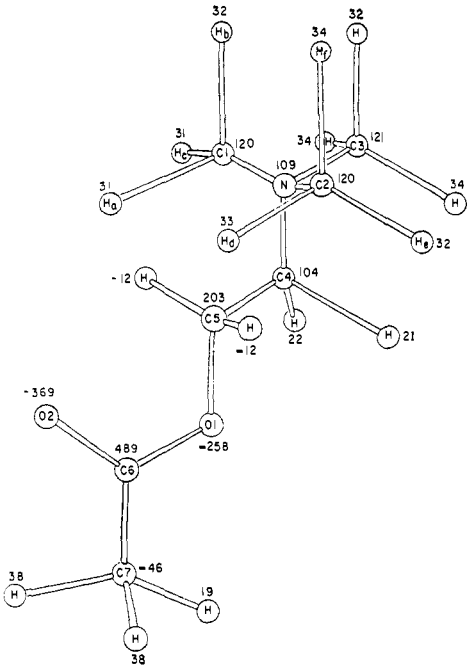

Figure 5. Net atomic charges ($\times 10^3$) of {180°, 180°} conformer of acetylcholine.

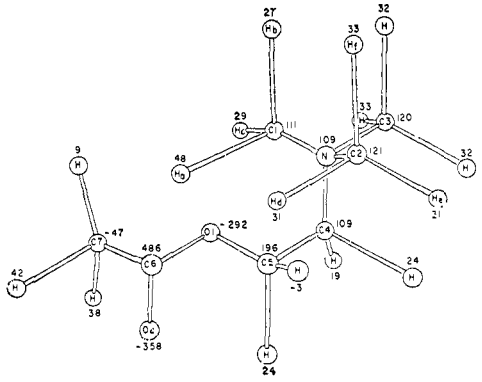

Figure 6. Net atomic charges ($\times 10^3$) of {40°, 180°} conformer of acetylcholine.

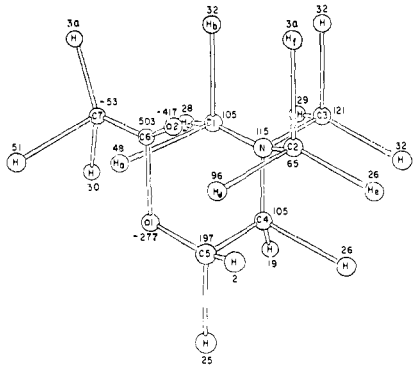

Figure 7. Net atomic charges ($\times 10^3$) of {50°, 270°} conformer of acetylcholine.

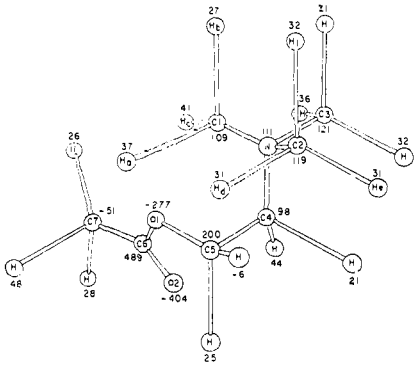

Figure 8. Net atomic charges ($\times 10^3$) of {50°, 50°} conformer of acetylcholine.

For both τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)), the synclinal conformations are calculated to be more stable than the other accessible conformations; *cf.* Figures 3 and 4. An understanding of the forces stabilizing the synclinal positions of both torsional angles can be developed in terms of intramolecular hydrogen bonding. Intramolecular hydrogen bonding is most commonly found in circumstances involving an A–H···X structure, where A and X are both electronegative with respect to hydrogen; the structure is stabilized by a combination of Coulomb, van der Waals, and charge-transfer forces.[22] In C–H bonds, the bond polarity is ordinarily very small, leading to little propensity for hydrogen bonding. However, for C–H bonds incorporated into a trimethylammonium cationic head, the electronegative quaternary nitrogen may effectively withdraw electrons from all the methyl C–H groups, activating the methyl hydrogens for hydrogen bonding. An accurate account of hydrogen bond energies is somewhat beyond the capability of the level of calculations presented herein, since van der Waals forces are not accommodated in the orbital approximation and since the semiempirical nature of the INDO methodology introduces considerable uncertainty in the calculated energies and energy differences. Nevertheless, a contribution from Coulomb forces should appear, and the corresponding trends in the relative energies and calculated charge distributions should be recognizable. This is significant in that the Coulomb energy is generally held to be the dominant attractive contribution to weak hydrogen bonds.[23]

The calculated net atomic charges for acetylcholine in the conformations {180°,180°}, {40°,180°}, {50°,270°}, and {50°,50°} are shown in Figures 5–8. The extended totally antiplanar {180°,180°} conformer serves as a convenient reference for a discussion of general aspects of the acetylcholine charge distributions, since secondary intramolecular interactions are minimal in this geometry. As expected, the positive charge associated with the quaternary nitrogen in the principal valence structure is delocalized over the entire cationic head, and the nitrogen atom due to its intrinsic electronegativity is in fact the least positive of the heavy atoms in the trimethylammonium group. The methyl carbons and hydrogens carry net positive charges of +0.12 and +0.03, respectively. The net atomic charges of all the atoms in the $-CH_2N(CH_3)_3$ cationic head grouping sum to +0.91. The ester oxygen and the carbonyl oxygen both carry net negative charges, and the high net positive charge of the carbonyl carbon is consistent with the electrophilicity attributed this atom from infrared and

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reaction kinetic[24] investigations. The ester linkage is not dipositive and, to the extent these calculated net atomic charges are correct, the hydrolysis of acetylcholine by acetylcholinesterase must be considered an exception to Pullman's dipositive bond theory of enzymatic hydrolysis.[25]

The electronic charge distributions of acetylcholine in other conformations corresponding to energy minima may be best understood in terms of perturbations on the distribution for the {180°,180°} geometry. In the {40°,180°} conformer the synplanar orientation of τ(O(1)–C(5)–C(4)–N) is marked by a charge redistribution involving the ester oxygen and a methyl hydrogen H_a. The negative net charge of the ester oxygen O(1) and the positive net charge of H_a are both increased from their respective values at {180°,180°}. The net charges in the cationic head still sum to +0.91, indicating there is charge polarization within the cationic head without significant charge transfer to other regions of the molecule. This polarization occurs in such a way as to increase Coulombic attraction between the ester oxygen and proximal methyl hydrogens, and correlates with a potential energy minimum. The $H_a \cdots O(1)$ distance at {40°,180°} is 1.88 Å, well within the van der Waals contact distance. These results are all consistent with an interpretation of the stabilization of {40°, 180°} in terms of intramolecular hydrogen bonding.

In the paired {50°,50°} and {50°,270°} forms of acetylcholine, the charge polarization effects characteristic of intramolecular hydrogen bonding show up for the trimethylammonium group and both the ester oxygen and the carbonyl oxygen. In {50°,50°}, the ester oxygen interaction involve two hydrogens of the same methyl group with the critical interatomic distances $O(1) \cdots H_a$ and $O(1) \cdots H_c$ being 1.98 and 2.65 Å, respectively. A methylene hydrogen of C(5) is also proximal to O(2) and carries a correspondingly high net positive charge. In the {50°,270°} geometry O(1) interacts with H_a at a distance of 1.98 Å and O(2) interacts with H_d at a distance of 2.1 Å. The net charges of the atoms of the cationic head in {50°,270°} sum to +0.92, indicating slight electronic charge transfer out of this region.

Thus, a characteristic feature of synplanar geometries associated with energy minima in the acetylcholine potential energy surface is charge polarization expected to be concomitant with the formation of weak intramolecular hydrogen bonds. The combination of energy stabilization, charge polarization, and intermediate distances within the van der Waals contact value indicate the synplanar stabilization of τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)) in calculations at the INDO level of approximation is due to intramolecular hydrogen bonding. More detailed theoretical and experimental considerations are in order on this point.

In comparing theory and experiment, the structural detail discussed above from a quantum theoretical viewpoint must of course be considered in the perspective of the physicochemical system. At room temperature many conformations will be thermally populated, and in solid and solution environmental effects are surely important. The hydrogen bonding interactions would be represented by averaged effects over rotating methyl groups and test calculations at several other methyl orientations indicate the description above remains valid. Intramolecular hydrogen bonding and the various conformations of {τ(O(1)–C(5)–C(4)–N), τ(C(6)–O(1)–C(5)–C(4))} would be influenced significantly by solvent polarity. The crystal geometry B and the related conformation A have both the acetoxy and acyloxy oxygen atoms in the interior of a roughly spheroidal structure with C–H bonds on the exterior. In solution these geometries would likely be favored by low solvent polarity, although acetylcholine as a cation has proved sparingly soluble in such solvents. Geometries such as D and G exposing one or both oxygens could be preferentially stabilized by highly polar, intermolecularly hydrogen bonding solvents.

The natural selection of acetylcholine as a chemical neurotransmitter has been attributed in part to its conformation flexibility, being adaptable to nicotinic, muscarinic, and possibly many other receptor sites. In light of the possible solvent effects on acetylcholine conformational stability, local biological environments could also serve to preferentially stabilize conformations complementary to specific receptor sites, and thus contribute to optimum biological function.

III. Summary and Conclusions

The INDO molecular orbital calculations reported herein reveal a number of energy minima in the acetylcholine potential energy surface with respect to the torsional angles τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)). As summarized in Table I, certain of the minima can be closely identified with the acetylcholine crystal geometry, the geometries implicated in muscarinic and nicotinic aspects of cholinergic action, and the geometry considered favorable for hydrolysis by acetylcholinesterase. The energy stabilization of synplanar values of both τ(O(1)–C(5)–C(4)–N) and τ(C(6)–O(1)–C(5)–C(4)) is attributed to intramolecular hydrogen bonding as evidenced by an electronic charge redistribution in a manner consistent with polarization effects expected for hydrogen bonding structures.

Thus a number of proposed structural aspects of the neurobiological action of acetylcholine can be understood and unified on the basis of quantum mechanical calculations, considering local minima in the potential energy surface and corresponding molecular electronic properties. Subsequent studies in this series will deal with the relationship of calculations on muscarinic and nicotinic agonists to those reported herein for acetylcholine.

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