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Quantum Mechanical Study of the Conformational and Electronic Properties of Acetylcholine and Its Agonists Muscarine and Nicotine

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SUMMARY

The conformational and electronic properties of acetylcholine, muscarine, and nicotine have been investigated by the quantum mechanical method of perturbative configuration interaction using localized orbitals. The results obtained in the field of preferred conformations, in particular in the case of acetylcholine, are in satisfactory agreement with experimental observations based on X-ray data. The distribution of the net electronic charges indicates, among other conclusions, the nearly neutral character of the quaternary nitrogen, the distribution of a large fraction (70–80%) of the formal positive charge of this nitrogen on the surrounding alkyl groups, producing a large cationic globe, and the near equivalence of the net total negative charges on the 2 oxygen atoms of acetylcholine. Altogether, the results do not favor a primordial role played by changes of conformation in the interactions of acetylcholine with different cholinergic nerve receptors, although some such changes may occur because of the appreciable flexibility of this molecule. They point rather to the importance in this respect of the electronic characteristics of the molecule. Thus, the involvement of the cationic globe in interactions with all cholinergic nerve receptors and that of the carbonyl and ester oxygens of acetylcholine in interactions with the nicotinic and muscarinic receptors, respectively, seems plausible.

INTRODUCTION

Acetylcholine (structure I) is the natural intercellular effector in nervous transmission systems, and its fundamental importance in this field has stimulated a great amount of experimental and, more recently, theoretical work on the structure of this molecule, both for its own interest and in comparison with related substrates of cholinergic systems. Generally, cholinergic receptors in the peripheral nervous system are divided pharmacologically into nicotinic and muscarinic, depending upon whether stimulation of the nerve junctions occurs by nicotine or by muscarine. Because of this dual specificity of the cholinergic receptors, there was a natural tendency to associate the activity of acetylcholine with two distinct structural or reactive features of this compound. Broadly speaking, these have generally been considered as consisting either of two distinct conformers (e.g., refs. 1–4) or of two different combinations of reaction sites on one (although possibly not too rigid) conformer (e.g., refs. 5 and 6).

Acetylcholine is a flexible molecule, and its structure, in particular its conformational possibilities, has attracted the at-

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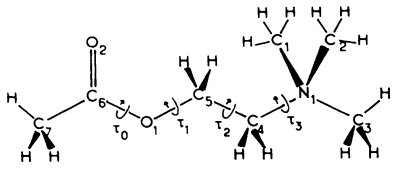
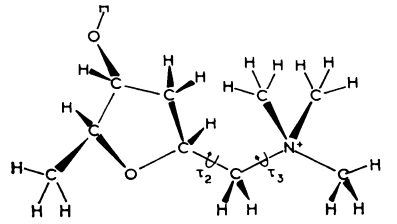
tention of theoretical investigators. Two studies have been carried out in this respect: one by an "empirical" procedure, in terms of van der Waals pairwise interactions between nonbonded atoms and threefold torsional potentials (7), and the other by a relatively simple molecular orbital quantum mechanical method, the extended Hückel theory (8). The two treatments led to different results, and, as will be seen from the forthcoming discussion, are both somewhat unsatisfactory. This situation prompted us to re-examine the problem theoretically with the help of a more refined quantum mechanical procedure, PCILO, perturbative configuration interaction using localized orbitals, developed recently in our laboratory (9, and references indicated therein), which has been used successfully in the study of conformational problems concerning a large number of biomolecules: the amino acid residues of proteins (10–13), nucleosides and nucleotides (14), steroids (15), disaccharides (16), retinals (17), and others.

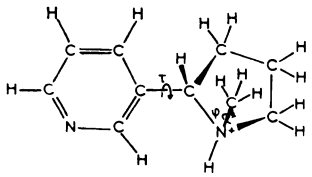
We have also extended our research to the study of the two acetylcholine agonists, muscarine (II) and nicotine (III). The first of these molecules has also been investigated empirically (18) and by the extended Hückel theory (8); the second, only by the extended Hückel theory (19).



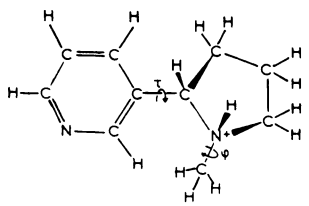
I



II



(a)



(b)

III

PROCEDURE

The designation PCILO stands for perturbative configuration interaction using localized orbitals. Details of the method are to be found in the original papers (9). Only its broad principles are outlined here. A larger summary has been presented elsewhere (13).

The method considers all valence electrons, and therefore involves simultaneous study of the σ- and π-electrons. It takes into account interelectronic repulsions and goes beyond the self-consistent field approximation in the calculation of the ground state energy, by incorporating an appreciable fraction of the correlation energy. Its fundamental idea is to choose a set of reasonable bonding and antibonding orbitals localized on the chemical bonds. Such a set may be constructed on a basis of hybridized atomic orbitals (χ_i), the bond orbitals being obtained as linear combinations of distinct hybrids taken two by two, each bonding orbital Φ_i being associated with an orthogonal antibonding orbital Φ_i^*:

$$\Phi_i = C_{i1}\chi_{i1} + C_{i2}\chi_{i2}$$
$$\Phi_i^* = C_{i2}\chi_{i1} - C_{i1}\chi_{i2}$$

A localized orbital representing a lone pair is described by a single hybrid orbital.

The bonding orbitals are then used to construct a fully localized Slater determinant. This determinant represents the 0-order wave function for the ground state of the system. The antibonding orbitals are utilized to build the excited states, and

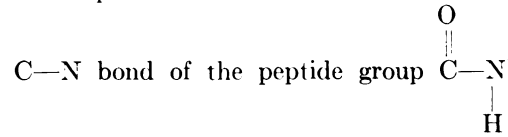
a configuration interaction matrix is considered to be constructed on such a basis of configurations. Then the lowest eigenvalue and eigenstate, i.e., the energy and the wave function of the ground state of the system, are obtained by a Rayleigh-Schrödinger perturbation expansion truncated after the third order.

As a technical simplification, the principal working hypotheses of the CNDO/2 procedure have been retained, in particular the hypothesis of complete neglect of differential overlap as well as the general parameterization of this procedure (20, 21).

The calculations have been carried out on an IBM-360-75 computer. The evaluated *energy differences* between the conformers are estimated to be significant within the limits of 0.5 kcal/mole.

The treatment of the molecules investigated was carried out along the following lines.

Acetylcholine (*I*). Although acetylcholine contains eight single bonds, there are only four important torsion angles (indicated as τ_1–τ_4 in structure I) and, in fact, only two essential such angles, τ_1 and τ_2.[1] Thus, in the first place the methyl groups of the quaternary nitrogen may be taken in staggered positions, the trimethylethylammonium backbone being (as it is in all known cholinergic molecules) antiplanar with $\tau_3 = \tau(C_5—C_4—N^+—C_3) = 180$ degrees. From our studies on dipeptides (10) we may also fix the C_7 methyl group with a C—H bond eclipsing the $C_6{=}O_2$ double bond. Moreover, because of the partial double bond character of the $C_6—O_1$ bond (similar to the partial double bond character of the



C—N bond of the peptide group), the torsion angle $\tau_0 = \tau(C_7—C_6—O_1—C_5) = 180$ degrees (5, 22). We are therefore left with the two essential torsion angles: $\tau_1 = \tau(C_6—O_1—C_5—C_4)$ and $\tau_2 = \tau(O_1—C_5—C_4—N^+)$. It is the study of these rotations on which our attention will be centered. They have been calculated with 20 degree increments, with the exception of the region near the global minimum, where the calculations have been refined using a 5 degree increment.

The geometrical input data for acetylcholine (bond lengths and bond angles) have been taken from the crystal structure of acetylcholine chloride (23), with the assumption of symmetry of the arrangement of the three CH_3 groups attached to N^+.

Muscarine (*II*). The two torsion angles taken into consideration define the rotations around the $C_{ring}—CH_2$ bond (designated τ_2 by analogy with acetylcholine) and around the $CH_2—N^+$ bond (τ_3). A staggered conformation (τ_3 = 60, 180, and 300 degrees) may, of course, be predicted for this last angle and, as will be seen later, is actually found. The geometrical input data (bond lengths and bond angles) for this molecule were taken from the crystallographic study of Jellinek (24) on muscarine iodide. Following the indications of this study, we have placed the ring methyl group on the same side as the chain $—CH_2—N^+(CH_3)_3$, while the OH group was placed on the opposite side.

Nicotine (*III*). Two rotational angles may be considered. One concerns the methyl group of the pyrrolidine ring (angle φ), although it is expected that this group is staggered with respect to the ring. The essential torsional angle τ defines the mutual arrangement of the two rings, pyridyl vs. pyrrolidine. We have varied simultaneously the two angles and have confirmed, as will be seen shortly, that the stable conformations correspond to the staggered arrangement of the methyl group. An ambiguity remains, however, concerning the mode of attachment of this methyl group to the pyrrolidine ring. Two possibilities are available a priori (IIIa and IIIb), corresponding to the *trans* and *cis* arrangements of the methyl group with respect to the pyridine ring, and in the absence of decisive evidence we have carried out calculations for both forms. The geometrical input data for this

[1] The torsion angle τ of the bonded atoms *A—B—C—D* is the angle between the planes *ABC* and *BCD*. Viewed from the direction of *A*, τ is positive for clockwise and negative for counterclockwise rotations. The value τ = 0 degrees corresponds to the planar-*cis* arrangement of the bonds *AB* and *CD*. Values of τ = 0, 60, 120, and 180 degrees are termed *syn*-planar, *syn*-clinal, *anti*-clinal, and *anti*-planar, respectively).

molecule correspond to standard values of bond lengths and angles.

RESULTS AND DISCUSSION

Acetylcholine. The conformational energy map of acetylcholine (*iso*energy curves as a function of τ_1 and τ_2) constructed by our quantum mechanical PCILO calculations is presented in Fig. 1. There is a global minimum situated at τ_1 = 180 degrees and τ_2 = 60 degrees, which, together with the adopted values of τ_0 = τ_3 = 180 degrees, corresponds to a structure which may conventionally be described by the symbol TTGT, underlying the *gauche* conformation of the O_1 and N^+ atoms and the *trans* arrangement of the remaining atoms of the backbone. A local minimum, situated about 1 kcal/mole above the global one, is found at τ_1 = 120 degrees and τ_2 = −80 degrees. [By symmetry, there are of course identical minima at (τ_1, τ_2) = (−180, −60 degrees) and (−120, 80 degrees); which correspond to enantiomorphs.] It may usefully be stressed that around these minima, and in particular around the global one, there are large plateaus of low energy, as illustrated by the relatively broad contour of the 1 kcal/mole *iso*energy curve. The minima, and in particular the global one, are therefore associated with a considerable flexibility, especially as concerns the variation of τ_1.

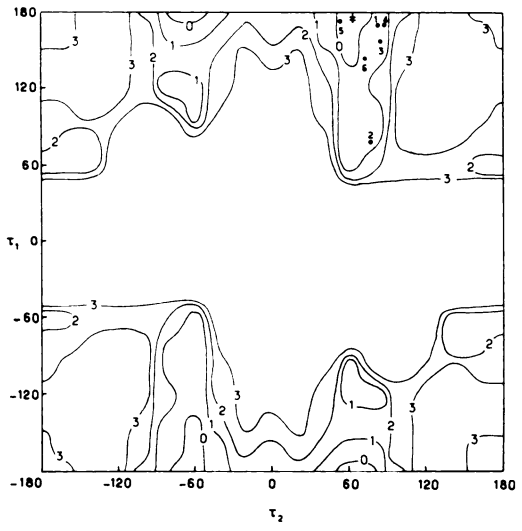


FIG. 1. *Conformational energy map of acetylcholine*
*Iso*energy curves in kilocalories per mole with respect to the global minimum (±), taken as zero energy. Shown are experimental conformations (●) in crystals of (*1*) acetylcholine chloride (23), (*2*) acetylcholine bromide (25), (*3*) lactoylcholine iodide (26), (*4*) acetyl-α-methylcholine (26), (*5*) dimethylphenylpiperazine (26), and (*6*) muscarine iodide (24).

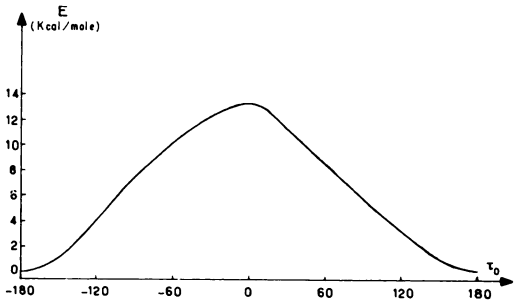


FIG. 2. *Energy variation for rotation around* τ_0 *of acetylcholine.*
τ_1 = 180 degrees; τ_2 = 60 degrees.

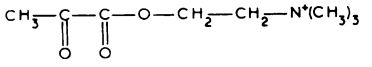
Before confronting these results with the available experimental evidence it may be useful to compare them with the previous theoretical studies, mentioned above. The "empirical" calculations of Liquori *et al.* (7) led to the prediction of *four* highly localized energy minima, all of them no more than 0.7 kcal/mole above the lowest among them. The most stable conformation corresponds, following those computations, to the *totally extended form*, followed by one in which the O_1 atom is *trans* to the N^+ atom. Our most stable conformation is only the third such on their scale.

The results of the extended Hückel theory have been obtained by computing individually the variations in energy corresponding to each torsion angle studied. The results lead to a preferred conformation associated with τ_1 = 180 degrees and τ_2 = 80 degrees and thus are close, from that point of view, to the most stable conformation found in our calculations. However, Kier (8) found that the carbonyl group is *free* to rotate 60 degrees to either side of the *planar-cis* arrangement with respect to the O_1—C_5 bond, i.e., that the torsion angle τ_0 corresponds to a constant value of energy between 120 and 240 degrees. This result led Kier to postulate two conformers with

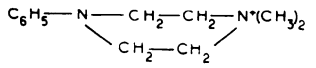
similar values of τ_1 and τ_2 but different values of τ_0 (4). Before engaging in further discussion, we may already say that our own computations disagree with Kier's results concerning the shape of the τ_0 rotational curve. The curve obtained by the PCILO method is given in Fig. 2. It shows the existence of a definite minimum at 180 degrees, corresponding to the planarity of the ester group, with the energy rising continuously upon departure from this arrangement. A 60 degree torsion would correspond to an increase in energy of about 4 kcal/mole.

We now turn to the available experimental results, which may shed some light on the real situation. These results come in the first place from X-ray studies on acetylcholine crystals and related compounds. The most significant from that point of view is, of course, the model compound of the calculation, acetylcholine chloride. The observed conformation (23) corresponds to $\tau_1 = 166.9$ degrees and $\tau_2 = 84.7$ degrees and, as can be seen from Fig. 1, falls very near our calculated global minimum, within the 1 kcal/mole *iso*energy curve. It corresponds to a *gauche* arrangement of the O—C—C—N^+ system. The same arrangement is also found in a number of closely related molecules—L (+)-lactoylcholine iodide (IV), with $\tau_1 = 157$ degrees and $\tau_2 = 85$ degrees (27); 1,1-dimethyl-4-phenylpiperazine (V), with $\tau_1 = 179$ degrees and $\tau_2 = 55$ degrees; and D(+)-acetyl-α-methylcholine (VI), with $\tau_1 = 170$ degrees and $\tau_2 = 90$ degrees (26)— and is considered by Sundaralingam (28) to be a common structural feature of nerve amines and phospholipids (see also ref. 22).

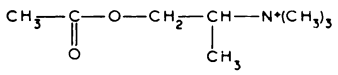
On the other hand, the crystal of acetylcholine bromide (25) corresponds to a significantly different conformation ($\tau_1 = 79$ degrees, $\tau_2 = 77$ degrees), which is a *gauche-gauche* arrangement. It can be seen, however, that although somewhat different from the compounds mentioned above, the conformation of acetylcholine bromide is still well within the 1 kcal/mole *iso*energy curve and



IV



V



VI

its energy therefore cannot be far above the energy of the *trans-gauche* arrangement. It is particularly gratifying to observe that NMR studies on acetylcholine bromide in D_2O have shown that this molecule assumes in solution the *trans-gauche* arrangement with $\tau_1 \approx 180$ degrees (29).

Altogether these experimental results confirm the significance of the quantum mechanical computations and differ from the predictions of the empirical ones, which proposed, as we have seen, that the most stable conformer of acetylcholine should be the all-*trans* one and that the *trans* and *gauche* conformers should possess little energy difference.

It may, however, be useful to indicate that acetylthiocholine and acetylselenocholine (compounds in which O_1 of structure I is replaced by S and Se) have been found to have the *trans* conformation of their N^+—C—C—S(Se) grouping [$\tau_1 = 129$ degrees and $\tau_2 = 171$ degrees in acetylthiocholine bromide, and $\tau_1 = 124$ degrees and $\tau_2 = 175$ degrees in acetylselenocholine bromide (30)] and that the *trans* conformation of acetylthiocholine is conserved in solution (31). In the absence of computations on these analogues, which we intend to investigate, we can only note from the quoted observations the perserverance of the preferred conformation, indicating probably a relatively high energy barrier between the *trans* and *gauche* arrangements.

Muscarine. The conformational energy map of this molecule is shown in Fig. 3. A single stable conformation is found, corresponding to $\tau_3 = 60$ or 180 or 300 degrees (staggered) and $\tau_2 = 60$ degrees. It is identical with the conformation found by Kier (8) (because of differences in the definition of $\tau_2 = 0$, the angle τ_2 for the stable conformation has the value of 180 degrees in Kier's

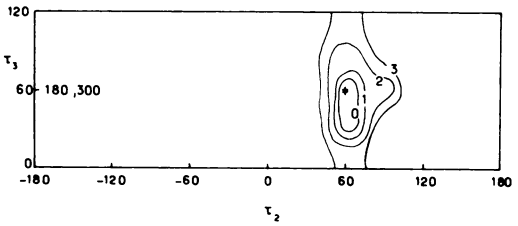


FIG. 3. *Conformational energy map of muscarine.*

*Iso*energy curves in kilocalories per mole with respect to the global minimum (±), taken as zero energy.

paper) and corresponds precisely to the experimental conformation (24). No appropriate comparison is possible with the results of the empirical computation (which seem to predict three local minima), because of difficulties in following the presentation of its results (compare Fig. 2 and Table 3 of ref. 18).

Nicotine. The conformational energy map of nicotine in its two calculated forms is shown in Fig. 4. In both forms there are two distinct regions of stability, which correspond to a staggered position of the methyl group of the pyrrolidine ring and are centered around 100–120 degrees and −40 to −80 degrees for the rotational angle τ, between the pyridyl and pyrrolidine rings. However, while the global minimum of form IIIa corresponds to $\tau = -40$ degrees and its secondary minimum to $\tau = 120$ degrees (at about 1 kcal/mole above the global one), the reverse is true for form IIIb, in which the global minimum occurs at $\tau = 100$ degrees and the secondary minimum (1 kcal/mole above) at $\tau_2 = -80$ degrees. Altogether the calculations predict form IIIa to be about 4 kcal/mole more stable than form IIIb, a result which seems to be in qualitative agreement with the conclusions of Craig *et al.* (32). Our results thus differ somewhat from those of the extended Hückel calculation of Kier (19), who studied the enantiomorph of form IIIa and also found two minima at similar coordinates, but estimated them to be equivalent in energy.

The experimental data (26) seem to be in relative agreement with the quantum-mechanical computations indicating the angle $\tau = -62$ degrees.

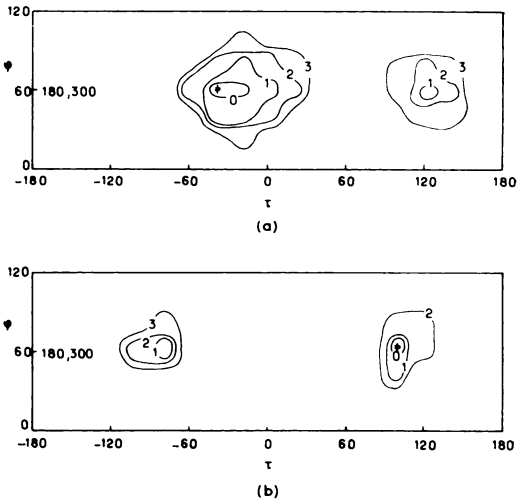


FIG. 4. *Conformational energy map of nicotine (IIIa and IIIb).*

*Iso*energy curves in kilocalories per mole with respect to the global minimum (±), taken as zero energy.

The principal general conclusions which can be drawn from this review of the theoretical results and their comparison with the available experimental evidence at this stage of our discussion are the following: (a) Acetylcholine has more conformational flexibility than muscarine or nicotine, (b) The flexibility of acetylcholine stems, however, from rotations around τ_1 and τ_2 (in particular around τ_1) and not, as claimed by the extended Hückel theory, from rotation around τ_0, (c) The *gauche* arrangement of the esteric O_1 atom with respect to the N^+ atom appears to be a preferred conformation in acetylcholine, the *trans* arrangement being approximately 3 kcal less stable, (d) Muscarine is the least flexible of the three compounds studied, being associated with a single and rather restricted minimum energy zone. Nicotine has two local minima, which, however, are not equivalent, one being about 1 kcal/mole more stable than the other.

With these results in mind we may consider somewhat more closely the different proposals related to the muscarinic and nicotinic receptors of cholinergic systems. It is obvious, of course, that since the calculations have been made for molecules in a a vacuum the results must be interpreted

with caution; however, the relationship seems worth exploring.

First, it is apparent that our theoretical results do not particularly favor the hypothesis that two fundamentally distinct conformations of acetylcholine interact with the two types of receptors. One global minimum, in whose vicinity the essential experimental conformations under investigation fall, has been found for this molecule, with the exception of acetylcholine bromide, which, however, reverts to this global minimum conformation in solution. When the appropriate rotational angles of muscarine and nicotine are plotted on the acetylcholine conformational energy map, they also fall near the global minimum of acetylcholine. It is true, however, that the low energy zone (less than 1 kcal/mole) located around the global minimum is a large one, permitting much flexibility.

In view of this situation we may then center our attention on the proposals which relate the dual receptivity of acetylcholine to distinct structural or reactive features of this compound. Two major proposals have been made recently in this respect. One, by Chothia (5), distinguishes two "sides" in acetylcholine, the methyl side and the carbonyl side, the first one activating the muscarinic receptors and the second one the nicotinic receptors. In more detail, the essential structural features of the muscarinic agonists responsible for their interaction with the receptor are, according to Chothia, the quaternary nitrogen group and the terminal methyl group (C_7 in acetylcholine), while the essential structural features of the nicotinic agonists are the quaternary group and the carbonyl group. The second proposal, recently presented extensively by Beers and Reich (6), also considers the dual receptor action to be due to interactions based on two different combinations of functional groups in the transmitter molecule. According to these authors, in the muscarinic series the functional groups which determine interaction are a quaternary ammonium grouping, the ester oxygen, whose lone pair of electrons can act as an acceptor for hydrogen bonding, and the terminal CH_3 group; in the nicotinic series the functional groups are again the quaternary ammonium center and the carbonyl oxygen, which also acts as an acceptor for hydrogen bonding.

The two proposals are therefore extremely similar with regard to nicotinic activity, which both suggest is determined by the combined action of the N^+ cationic center and the carbonyl group [the same concept was also proposed by Kier (4)]. They differ somewhat in their conception of the muscarinic agents. In particular, while Beers and Reich attach great importance to the role of the esteric oxygen in this respect, no special role seems to be attributed to this atom by Chothia.

In an effort to shed some light on the validity of these proposals, we may consider two structural features of the molecules studied here which may possibly play a significant role in the correlation proposed. These are the interatomic distances among the complementary active groups (the aforementioned proposals being generally associated with restrictions on these distances) and the distribution of electronic charges, which may, of course, play a dominant role in the chemical action involved.

Calculation of the interatomic distances associated with the most stable conformations predicted for acetylcholine, muscarine, and nicotine indicates a theoretical distance of 3.02 A between N^+ and the esteric oxygen atoms (against the experimental crystal value of 3.26 A) and of 4.93 A between N^+ and the carbonyl oxygen atom in acetylcholine. In muscarine, the calculated N^+—esteric oxygen distance is 2.91 A (compared with the experimental value of 3.07 A). Finally, the calculated distance between the two nitrogen atoms in nicotine is 4.3–4.7 A. These results point in particular to the close similarity, mentioned frequently above of the N^+—esteric oxygen distances in acetylcholine and muscarine and, from the usual pharmacological point of view, would suggest that these two sites play a role in muscarinic nerve receptors. The N^+—N distance in nicotine is close to the N^+—carbonyl oxygen distance in acetylcholine.

Another hypothesis, put forward by Chothia and Pauling (26), proposed that the conformation of cholinergic molecules for binding to nicotinic receptors involves a planar acetoxy or aromatic ring and a

charged methylated ammonium group, with a variable distance between the two but with the N^+ atom about 1.3 A above the plane of the acetoxy or aromatic group. Our computations indicate that this out-of-plane distance is 1.1 A in acetylcholine and 0.9 A in nicotine.

Some more striking and original conclusions are suggested by the distribution of the electronic charges in the molecules studied (Fig. 5). However, the charges shown in Fig. 5 are total net electronic charges (i.e., a summation of the net σ- and π-charges, where "net charges" denotes an excess or deficit at each atom in relation to the number of electrons the atom would possess in an isolated state). Thus they differ from the usual representations, in which σ- and π-electrons are depicted separately (except, of course, for those obtained by the extended Hückel theory).

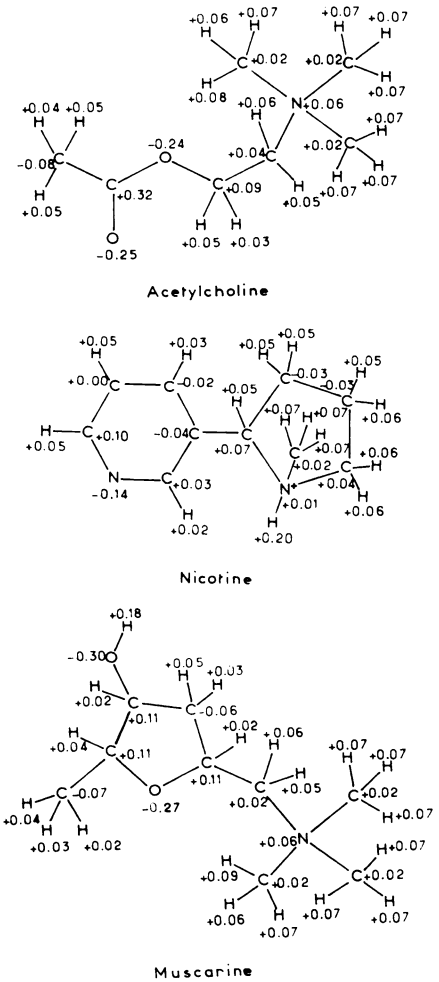


FIG. 5. *Net total (σ + π) electronic charges in acetylcholine, muscarine, and nicotine*

The minus sign means net negative charge; the plus sign means net positive charge (in electron units).

Examination of the charge distributions of Fig. 5 leads to the following essential conclusions.

1. The N^+ atom is in fact nearly neutral.

2. In both acetylcholine and muscarine a large part (70%) of the formal positive charge is distributed among the three attached methyl groups. These three methyl groups thus form a kind of large ball of spread-out positive electricity to which the designation of a hydrophilic cationic center is particularly appropriate. The remaining fraction of the positive charge seems to be concentrated on the two CH_2 groups of acetylcholine or on the CH_2 group and the adjoining ring carbon of muscarine, further enlarging the dimension of the cationic moiety of these molecules. In nicotine, about 80% of the formal positive charge is distributed on the periphery of the pyrrolidine ring and the attached methyl group.

3. The carbonyl and the ester oxygens of acetylcholine bear very similar net total charges. Both atoms carry an excess of about 0.25*e* and are thus both negatively charged. The intermediate carbon atom carries an appreciable excess of positive charge.

4. A similar situation concerns the ring and hydroxyl oxygens of muscarine. Both are negative and carry a somewhat larger negative charge than the 2 oxygen atoms of acetylcholine.

5. In nicotine, the pyridine nitrogen carries, as expected, an excess negative charge; however, this excess ($-0.145e$) is much smaller than suggested by the extended Hückel computations. It is smaller than the excesses on the oxygen atoms mentioned above.

These results underline the similarities in the electronic constitutions of the compounds studied and thus help to envision common mechanisms for their interaction with cholinergic nerve receptors. The three compounds possess similar cationic centers

or globes, with small fractional positive charges widely distributed upon the peripheral carbon and hydrogen atoms. All possess one or two anionic centers some distance away toward the other end of the molecule. The equivalence of the total electronic charge of the 2 oxygen atoms of acetylcholine suggests that both participate in interactions with nerve receptors, which makes tempting the proposal by Beers and Reich that assigns one oxygen to interaction with muscarinic receptors and the other to interaction with nicotinic receptors. However, the mere value of the net charge is not a sufficient measure of the chemical reactivity of an atom or molecule, because such reactivity depends on the dynamic properties (such as polarizability) of the system.

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