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Relatio
An EHT Re-Examination of Acetylcholine

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The results of EHT calculations on the most stable conformations of the neurotransmitter molecule acetylcholine (ACh) are reported. These results are compared with those obtained with other semiempirical quantum mechanical methods CNDO/2, INDO and PCILO, and with those obtained by the classical partitioning of the energy method (PEM). From this comparison it becomes evident a wide agreement between the results of PCILO, EHT and PEM, all these methods allowing accessibility to discrete regions in conformational space.

Key words: Acetylcholine, electronic structure of ~

The allowed conformations of the neurotransmitter molecule acetylcholine (ACh) $(CH_3)_3N^+—CH_2—CH_2—O—C(=O)CH_3$ has been the subject of extensive experimental [1–4] and theoretical [5–10] investigations.

At the present time there is a basic theoretical agreement on the flexibility of this substrate while the differences in the details of the results are critically examined in many laboratories.

A satisfactory agreement exists among a number of features [6, 9, 10] derived with the classical semiempirical procedures of the partition of the energy in physically relevant contributions (PEM) [11] and with the quantum mechanical procedure PCILO [12]. Notwithstanding the prediction of PCILO for a low barrier about the torsion angle ψ_2 ($N—C_\alpha—C_\beta—O$) (Fig. 1), a feature in common with the results [7–10] of INDO [13] and CNDO/2 [14] quantum mechanical procedures, the predictions about position and energy differences are in better agreement as derived with PCILO and PEM than with PCILO and INDO or CNDO/2 techniques.

Among the available theoretical investigations, that carried out with the EHT [15] procedure has been apparently less successful in predicting the allowed conformations as supported by the experimental data.

It turns out in fact from the analysis of Kier [5] on ACh with the EHT method that for the torsion angles ψ_1 ($C_\alpha—C_\beta—O—C$) and ψ_2 only one conformation, later found experimentally, in solution [3] and in the crystal of the chloride [4], but not in that of the bromide [2], should be allowed on energy grounds.

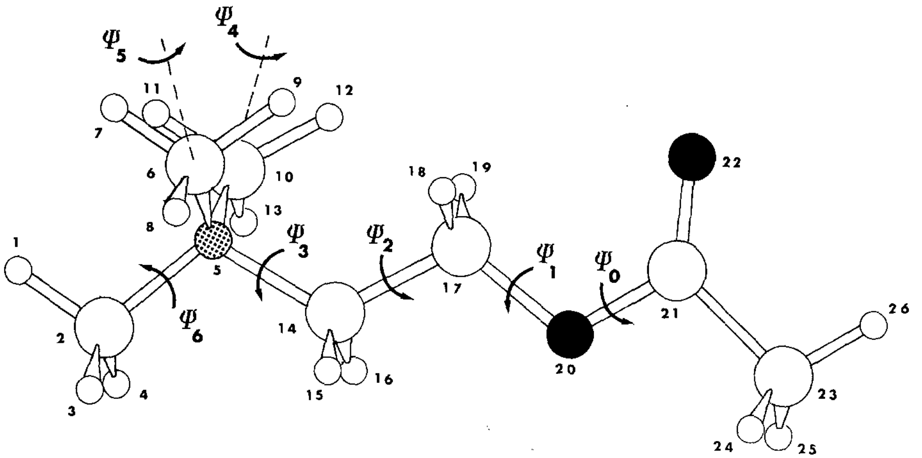

Fig. 1. Schematic view of the ACh molecule corresponding to the starting fully extended *TTTT* conformation. Labels for the conformation about the skeletal bonds are in the order from ψ_0 to ψ_3. Torsions are measured from the antiplanar conformations and are positive for counterclockwise rotations, $\psi_0(C_{17}–O_{20}–C_{21}–C_{23})$, $\psi_1(C_{14}–C_{17}–O_{20}–C_{21})$, $\psi_2(N_5–C_{14}–C_{17}–O_{20})$, $\psi_3(C_2–N_5–C_{14}–C_{17})$, $\psi_4(H_{13}–C_{10}–N_5–C_6)$, $\psi_5(H_8–C_6–N_5–C_{10})$, $\psi_6(H_1–C_2–N_5–C_{14})$

But as Kier carried out the analysis by varying the torsional angles individually and with large increments a more complete analysis based on results derived at the same level of complexity of the other procedures has been considered to be appropriate.

This, as well as the successful computations on a number of charged molecules carried out with the EHT and PEM techniques [16, 17] prompted us to undertake a re-examination of the allowed conformations of the ACh molecule with the EHT procedure.

A comparison of a number of C_α, C_β unsubstituted derivatives of choline indicates that bond lengths and angles used in the previous PEM analysis [6] were accurate enough for the purpose of this investigation (Table 1), therefore we used them also in the present investigation, however a number of peculiarities derived there from refinements on a group of seven torsion angles have been incorporated in the starting conformation of ACh.

It was shown in that paper that by labelling with *T* an extended antiplanar and with *G* a folded ($\sim 120°$ of torsion from antiplanarity) synclinal conformation about a bond and by taking into consideration the sequence of the torsion angles from ψ_0 to ψ_3 (Fig. 1), four conformations corresponding to the *TTTT*, *TGTT*, *TTGT* and *TGGT* ones, turned out to be grouped in the rather narrow energy range of 0.8 kcal/mole. After refinement of these four conformations it was found for two of them (*TTTT* and *TGTT*) an almost exactly antiplanar conformation about ψ_3 (C—N—C_α—C_β) but a deviation from antiplanarity of about $-6°$ for the other two (*TTGT* and *TGGT*)[1]. Furthermore the methyls on the onium

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[1] It is interesting to remark that in the most refined structure of ACh chloride [4] the torsion about ψ_3 as measured from the sequences $C_2–N_5–C_{14}–C_{17}$, $C_6–N_5–C_{14}–C_{17}$, $C_{10}–N_5–C_{14}–C_{17}$ are respectively -9, -8, -6 degrees. The mean value of $-7.5°$ is therefore in good agreement with the theoretical predictions.

Table 1. Values for bond lengths and angles as derived from the crystal structure of a number of choline derivatives with unsubstituted $-CH_2-CH_2-$ group

Bond	C_2-N_5	C_6-N_5	$C_{10}-N_5$	N_5-C_{14}	$C_{14}-C_{17}$	$C_{17}-O_{20}$	$O_{20}-C_{21}$	$C_{21}-O_{22}$	$C_{21}-O_{23}$
Mean values[a]	1.51	1.51	1.53	1.51	1.53	1.46	1.34	1.22	
ACh–Br[b]	1.50	1.52	1.52	1.54	1.52	1.50	1.32	1.25	1.55
This paper	1.52	1.52	1.52	1.54	1.52	1.50	1.32	1.25	1.55
Angles	$C_2-N_5-C_6$	$C_2-N_5-C_{10}$	$C_2-N_5-C_{14}$	$C_6-N_5-C_{10}$	$C_6-N_5-C_{14}$	$C_{10}-N_5-C_{14}$	$N_5-C_{14}-C_{17}$	$C_{14}-C_{17}-O_{20}$	$C_{17}-O_{20}-C_{21}$
Mean values[a]	109	109	111	109	107	110	117	108	114
ACh–Br[b]	106	–	111	109	–	109	118	113	115
This paper	109	109	110	109	110	110	114	113	115

[a] Mean values are derived from the data of Refs. [2, 4, 21, 22, 23, 29].

[b] Values for acetylcholine bromide are derived from Ref. [2].

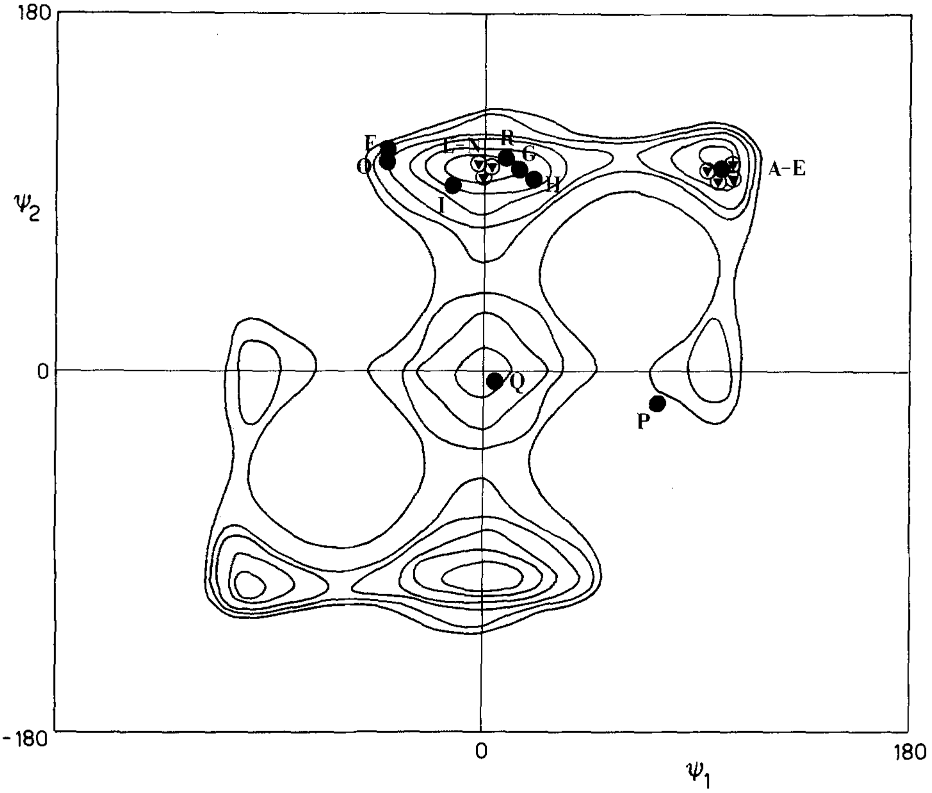

Fig. 2. EHT energy map of ACh in the undistorted $\psi_3 = 0^\circ$ conformation. Energy contour lines at constant increments of 0.5 kcal/mole. ● ratherly accurately defined conformation ▼ poorly defined conformation, Letters are in agreement with Table 3

group have been found rotated, almost independently from the rotations of the skeletal bonds, about -7, 7 and 0 degrees around ψ_4, ψ_5 and ψ_6 respectively (Fig. 1).

In the starting conformation of ACh ψ_4, ψ_5 and ψ_6 have been therefore fixed at the above mentioned values, while due to the rigidity of the ester bond [2] ψ_0 has been fixed to the antiplanar position.

To gain insight on the dependence of the quantum mechanical predictions from the torsion about ψ_3, two distinct sets of computations have been carried out, the first with ψ_3 fixed at 0°, the second one with ψ_3 at -5°.

For the two cases the conformational energy has been derived as a function of the two parameters ψ_1 and ψ_2 increased at constant steps of 20° degrees, by using in the EHT procedure the parametrization of Hoffmann and Imamura [18], slightly different from the previous one of Kier [5]. The most symmetrical ($\psi_3 = 0^\circ$) map is reported in Fig. 2, while in Table 2 are summarized the energy of the minima corresponding to both situations.

The lowest energy *TTGT* conformation already predicted by Kier is confirmed but the full set of the allowed conformations falls in a rather short energy range, well comparable with the PEM or PCILO ones, and therefore

Table 2. EHT energy differences (kcal/mole) for ACh for the undistorted $\psi_3 = 0°$ and for the distorted $\psi_3 = -5°$ model. Energies are computed from the global minimum *TTGT* at $\psi_3 = -5°$ fixed at zero

Conformation	Model	
	$\psi_3 = 0°$	$\psi_3 = -5°$
TTTT	1.6	1.6
TGTT	2.4	2.5
TTGT	0.4	0.0
TGGT	1.1	0.7
*TG*TT*[a]	2.4	2.1
*TTG*T*	0.4	1.1
*TG*G*T*	1.1	1.9

[a] Starred symbols for negative rotations.

further support is gained to the idea of flexibility of ACh and to the usefulness of the above schematization for the allowed conformations of ACh.

But finer details are likewise worthy of mention. The energy difference of about 0.8 kcal/mole computed for the conformations *TGGT* ($\psi_3 = 0°$) and *TG*G*T* ($\psi_3 = -5°$) (Table 2) is related to a variation of 0.06 Å (from 1.86–1.80 Å) of the interatomic distance between atoms H(9)...H(18) and to a like variation of 0.06 Å (from 2.53–2.47 Å) between atoms C(6)...H(19). Likewise the greatest energy variation computed for equivalent pair of conformations is that between *TGGT* ($\psi_3 = -5°$) and *TG*G*T* ($\psi_3 = -5°$). The value of 1.2 kcal/mole corresponds to a change in the interatomic distances of the mentioned pairs of about 0.14 Å. We are confident therefore that inaccuracy in bond lengths, of the order of those derived from crystal structures determination, that are from three to five times smaller, and of few degrees in the bond angles are likely to bear no significant role on the picture emerging from the present computations.

Further the energy barriers that have been found in some instances to be greater than the experimental ones [15] are in ACh for rotations about ψ_2 in the $-120° < \psi_2 < 120°$ region lower than those predictable from PEM computations. This result is in agreement with the INDO [7], PCILO [8] and CNDO/2 [10] predictions even if the last three mentioned procedures allow for greater flexibility about this torsion angle, clearly a point open to further investigation.

Anyhow in agreement with former observations the EHT computations allow for greater accessibility in conformational space [18], a feature in common to other quantum mechanical procedures [19].

The computed maps account for the distribution of the experimental points (Fig. 2) in a rather satisfactory way, to the conformation *TTGT* of the lowest energy minimum correspond, among the others, the observed conformations of ACh in the crystal of chloride [4] and in solution [3], the conformation of carbamylcholine[2] (Carbachol) in solution [20] and the conformation of

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[2] The EHT and CNDO/2 conformational energy maps of Carbachol are quite similar to that of ACh. The ACh maps can be therefore profitably used to interprete the allowed conformations of the group of the carbamylcholine derivatives with unsubstituted C_α—C_β hydrogen atoms.

Table 3. Conformations of a number of choline derivatives. Capital letters are in agreement with those of Fig. 2

		ψ_1	ψ_2	Ref.
A	Acetylcholine bromide	−101	−103	[24]
B	Palmitylcholine iodide	*G*	*G*	[25]
C	Stearylcholine iodide	*G*	*G*	[25]
D	Stearylcholine bromide	*G*	*G*	[26]
E	Laurylcholine iodide	*G*	*G*	[27]
F	L-α-glycerylphosphorylcholine I	42	−107	[28]
G	Dimethyl-5,6-phenylcarbamylcholine bromide	17	99	[21]
H	L-lactoylcholine iodide	− 23	− 95	[24]
I	Acetylcholine chloride	13	− 95	[4]
L	Acetylcholine	*T*	*G*	[3]
M	Carbamylcholine	*T*	*G*	[20]
N	L-α-glycerylphosphorylcholine $CdCl_2 \cdot 3H_2O$	*T*	−130	[30]
O	L-α-glycerylphosphorylcholine II	− 40	105	[28]
P	Phenyl-4-carbamylcholine bromide	74	− 18	[23]
Q	Carbamylcholine bromide	6	− 2	[22]
R	Methyl-4-carbamylcholine bromide	8	107	[29]

dimethyl-5,6-phenyl-Carbachol in the crystal of the bromide [21], the *TGGT* conformation 0.7 kcal higher has been found in the crystal of ACh bromide [2], the fully extended *TTTT* one 1.2 kcal higher in the crystal of Carbachol bromide [22] and finally the less favoured *TGTT* one in the crystal of phenyl-4-Carbachol bromide [23].

In conclusion the EHT predictions compare rather well with those of PEM and PCILO, and the useful schematization previously made [6] of four preferred regions is well supported.

Moreover, notwithstanding the differences in the relative order of the minima, the allowed conformations are always grouped in a range of about 2 kcal/mole and under varying field of forces any one of them can be easily preferred against the others.

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