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Conformational Analysis of d-Tubocurarine: Implications for Minimal Dimensions of its Binding Site within Ion Channels

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Abstract. All the minimum-energy conformations of d-tubocurarine were calculated by the method of molecular mechanics. The energy was minimized from 413 closed forms of the 18-member ring. The set of minimum-energy conformations includes 10 forms with energies less than 6 kcal/mol from the most stable one. Among the four lowest minimum-energy conformations, two forms correspond to those known from X-ray studies, whereas two conformations were not detected experimentally earlier. The flexibility of d-tubocurarine was estimated by calculating six paths of interconversion between the four lowest minimum-energy conformations. Using a molecular graphics technique, it was found that the most extended minimum-energy conformation of d-tubocurarine may fit in an ion channel of a rectangular profile of 8.7 × 11.2 Å, while one tetrahydroisoquinoline head may fit a profile as small as 6.9 × 11.0 Å. A possible model of d-tubocurarine location within the ion channel of the neuronal nicotinic acetylcholine receptor is suggested.

Key words: Tubocurarine—Conformation—Ion channel—Mechanism of blockade—Acetylcholine receptor

Introduction

d-Tubocurarine (TC) blocks the transmission of nerve impulses at the neuromuscular junction. Initially, the physiological action of TC was connected with the blockade of the acetylcholine recognition site of nicotinic acetylcholine receptor (AChR) (Michelson & Zeimal, 1973). In the last two decades, it was shown that TC may also block the ion channels activated by acetylcholine (Manalis, 1977; Katz & Miledi, 1978; Ascher, Large & Rang, 1979; Colquhoun, Dreyer & Sheridan, 1979; Rang, 1982), Ca^{2+} (Nohmi & Kuba, 1984), and glutamate (Yamamoto & Washio, 1979). Selyanko et al. (1988) have shown that the blockade of the open ion channel of AChR is the single mechanism of action of TC in the neurons of sympathetic ganglia. These findings stimulate speculation on the modes of TC binding within ion channels and raise the question of the conformational properties of TC.

X-ray crystallographic studies have revealed two different conformations of TC (Codding & James, 1973; Reynolds & Palmer, 1976). However, it remains unclear whether other conformations of TC are possible. In this work, using a molecular mechanical method, we have calculated all minimum-energy conformations (MEC) and the transition points between the lowest MEC of TC, evaluated minimal rectangular profiles of channels which would accommodate the lowest MEC of TC, and suggested a model of TC binding within the ion channel of the neuronal AChR.

Materials and Methods

Method of Calculations

The chemical structure of TC is shown in Fig. 1. We took up the protonated form of TC with the S-configuration of atom N1 corresponding to that in a crystal (Reynolds & Palmer, 1976).

Conformational analysis was carried out on an IBM PC/AT computer using the ZMM package, the latest version of a universal program for the molecular mechanical calculations (Zhorov, 1975). Conformational energy was minimized in the space of torsional angles and bond angles of atoms 1–36. Nonbonded and electrostatic interactions, torsional energy, the energy of bond

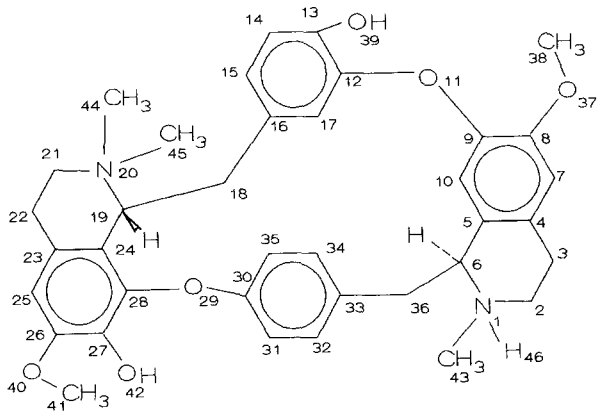

Fig. 1. Structural formulae of d-tubocurarine with atoms numbered as in the work of Codding and James (1973).

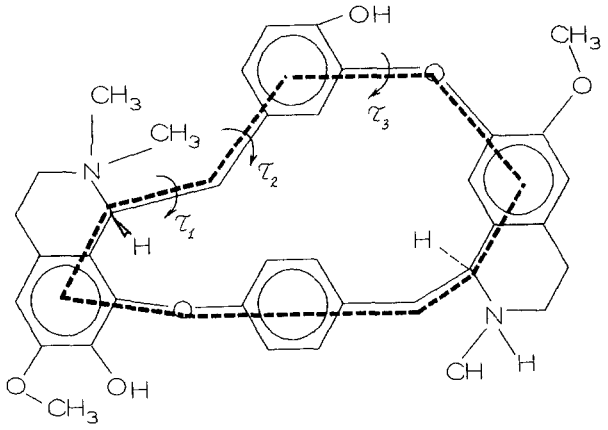

Fig. 2. The 9-membered virtual cycle, a model for the 18-membered main ring of d-tubocurarine.

angles deformation, and the loop closing potential were taken into account. The torsional energy was calculated to be $E_{tor} = (U/2)(1 - \cos 2\tau)$ for the bonds Ph–O, C_{sp2}–C_{sp2}, and $E_{tor} = (U/2)(1 + \cos 3\tau)$ for other bonds. The following barrier heights U (kcal/mol) were used: C_{sp3}–C_{sp3}, 3.0; C_{sp3}–C_{sp2}, 0; C_{sp2}–C_{sp2}, 20; N–C, 2.0; N^+–C, 3.0; O–C_{sp3}, 1.0; O–Ph, 4.0. Electrostatic interactions were calculated according to Coulomb's law. The CNDO/2 partial atomic charges were computed using a program by Maslov (1977) with the atomic coordinates found by the energy minimization from a crystallographic conformation of TC (Reynolds & Palmer, 1976). A value of 4.0 was used for the dielectric constant. Other details of calculations are described elsewhere (Zhorov & Govyrin, 1979; Zhorov et al., 1991).

The paths of conformational inversion between the minima were calculated using the procedure PATH of the ZMM package which is close to that described by Wiberg & Boyd (1972). The procedure PATH finds a torsion which deviates most in the two chosen minima, declares it to be the driven torsional angle or the reaction coordinate, calculates the shortest direction between the minima in the space of the generalized coordinates, and runs along this direction with a fixed step, thus generating a set of starting points. From each starting point, the energy is minimized with the driven torsion constrained at its starting value, the other torsional and bond angles being allowed to vary. Thus, a set of points characterizing the geometry and energy of the path is calculated, the maximum energy point indicating the transition state. A step of 0.2 radian between the starting points along the interminima direction was used.

Conformers were visualized using the Desk Top Molecular Modeling package (Oxford Electronic Publishing). To estimate the minimal-profile dimensions of a conformer, its wire-frame display was turned about three orthogonal axes to fit a minimal-size rectangle (Voitenco et al., 1991). The dimensions of space-filling models were obtained by adding 2.4 Å (doubled van der Waals radius of the hydrogen atom) to the width and height of the rectangle.

Results and Discussion

Minimum Energy Conformations and their Flexibility

A systematic search for the MEC of TC was made from the starting points calculated with the method of Go and Scheraga (1970). For an n-membered ring with given values of bond lengths, bond angles and $n - 6$ independent torsions, this method allowed us to calculate values of six dependent torsions corresponding to the ring-closing conformations. To find a complete set of the closed conformations of the n-membered ring, we calculated all the loop-closing values of the dependent torsions in each knot of the $(n - 6)$-dimensional grid of independent torsions. The main cycle of TC incorporates 18 bonds which make 12 independent torsions. Eight of these torsions are within the rigid aromatic cycles and, consequently, have definite values (0 or 180°). Two bonds in the para-position of the phenyl ring, O29–C30 and C33–C36, make one line. Consequently, the two corresponding torsions can be substituted by a single one which specifies the rotation of the fragment C28–O29–Ph–C36–C6 around the virtual bond O29–C36. This leaves only three independent torsions.

Figure 2 shows a nine-membered virtual cycle which models the main cycle of TC. A three-dimensional grid of the independent torsions τ_1, τ_2, τ_3 with 30° spacing (3^{12} knots) was constructed for this cycle. The procedure described by Go and Scheraga (1970) was carried out in each knot of the grid. Among virtual cycle closing conformations, we filtered only those in which the absolute values of τ(4-5-6-1) and τ(20-19-24-23) did not exceed 60°—the range providing a rough closure of the six-membered rings of tetrahydroisoquinoline fragments. Cyclohexene, a model for this ring, is known to adopt two half-chair MEC (*see* Dashevskiy, 1974, p. 188) with the values of torsional angles along the chain C*=C–C–C–C–C–C* being about 0, 20, −60, 60, −60, −20° or 0, −20, 60, −60, 60, −20°. Depending on the signs of the torsional angles τ(4-5-6-1) and τ(20-19-24-23) in the main ring closing conformation, corresponding sets were used as the starting points

Table 1. Energy (kcal/mol) and main torsional angles (degree) of the lowest minimum energy conformers of d-tubocurarine

Conformer				1	2	3	4	5	6	7	8	9	10
Energy				0.0	0.8	1.5	2.0	2.2	4.0	4.6	4.9	4.9	5.8
Torsion[a]													
10	5	6	36	−79	−78	−77	−42	−89	−50	−41	−92	−85	−43
5	6	36	33	57	62	6	−38	35	−34	−40	44	56	−35
6	36	33	32	87	55	−88	−61	−117	132	−60	−129	−91	−43
31	30	29	28	14	21	−14	4	−15	18	12	−3	83	−11
30	29	28	24	−109	−121	90	82	86	−127	74	76	−120	89
28	24	19	18	85	79	68	67	71	104	48	75	94	40
24	19	18	16	−147	−103	−149	−154	−151	−51	−160	−107	27	−107
10	9	11	12	−28	60	138	130	134	77	126	75	123	81
9	11	12	17	97	−120	−49	−44	−57	82	−43	61	−68	58
5	6	1	2	52	53	53	−42	58	−36	−42	61	57	−40
6	1	2	3	−60	−62	−61	65	−55	64	65	−52	−58	65
1	2	3	4	39	38	38	−53	26	−55	−53	18	31	−54
5	4	3	2	−11	−9	−9	21	−2	22	20	5	−5	21
24	19	20	21	−58	−59	−51	−52	−54	−66	36	−58	−66	45
19	20	21	22	57	61	63	62	62	40	−65	62	54	−66
24	23	22	21	6	3	8	9	8	−20	−19	4	−1	−13
17	16	18	19	54	170	45	38	47	−104	50	−70	−76	−42
20	21	22	23	−31	−33	−40	−41	−38	3	55	−35	−21	49
Distance (Å)													
N1 . . . N20				10.4	11.3	8.4	7.2	8.6	10.7	7.6	8.4	11.1	8.5

[a] The absolute values of the torsional angles in phenyl rings do not exceed 8°.

Table 2. Torsional angles (degree) of d-tubocurarine and trimethyl tubocurarine in crystallographic* and calculated conformations and Root Mean Square Deviations (RMSD) between the angles

Torsion				[a]	[b]	[c]	Conf. 1	[d]	Conf. 3
10	5	6	36	−75	−81	−80	−79	−79	−77
5	6	36	33	51	61	57	57	13	6
6	36	33	32	80	76	88	87	−90	−88
31	30	29	28	51	26	29	14	−20	−14
30	29	28	24	−132	−123	−122	−109	98	90
28	24	19	18	79	74	78	85	74	68
24	19	18	16	−149	−148	−142	−147	−145	−149
10	9	11	12	−13	−40	−24	−28	122	138
9	11	12	17	115	116	105	97	−20	−49
5	6	1	2	50	52	51	52	54	53
6	1	2	3	−54	−67	−61	−60	−66	−61
1	2	3	4	42	45	42	39	44	38
5	4	3	2	−22	−20	−18	−11	−16	−9
24	19	20	21	−44	−46	−53	−58	−50	−51
19	20	21	22	62	59	64	57	63	63
24	23	22	21	27	22	17	6	16	8
17	16	18	19	51	50	49	54	24	45
20	21	22	23	−51	−46	−43	−31	−43	−40
RMSD from									
[a]					10	9	14		
[b]						6	10		
[c]							7		
[d]									10

* Crystallographic conformations are denoted by [a](Sobell et al., (1972), molecule 1); [b](Sobell et al., 1972, molecule 2); [c](Reynolds & Palmer, 1976; [d](Codding & James, 1973)

for the torsions of six-membered rings. Thus, we filtered a total of 413 conformations of TC in which all rings are closed.

All these conformations were used as the starting points for the two-stage energy minimization. In the first stage, the phenyl rings and bond angles were kept rigid, the methoxy and hydroxy groups were eliminated, and electrostatic interactions were not taken into account. The minima found in the first stage were used as the starting points for energy minimization of the complete model of TC. In doing so, the starting values of 90, 90, 0, and 180° were assigned for the torsions C7–C8–O–Me, C27–C26–O–Me, C28–C27–O–H, and C12–C13–O–H, respectively. Minimization gave 39 conformers with energies in the range 0–31 kcal/mol; the 10 lowest energy conformers are given in Table 1. Subsequent calculations have shown that orientation of the hydroxy and methoxy groups and electrostatic interactions affect conformations of the main ring of TC only slightly.

Among the four lowest MEC (Fig. 3), conformations 1 and 3 correspond to the crystallographic structures of TC obtained by Reynolds and Palmer (1976) and by Codding and James (1973), respectively. Conformation 1 agrees also with the X-ray structure of O,O′,N-trimethyl-tubocurarine (Sobell et al., 1972). The root mean square deviation (RMSD) between calculated and experimental torsions varies from 7 to 14° (Table 2). Interestingly, the

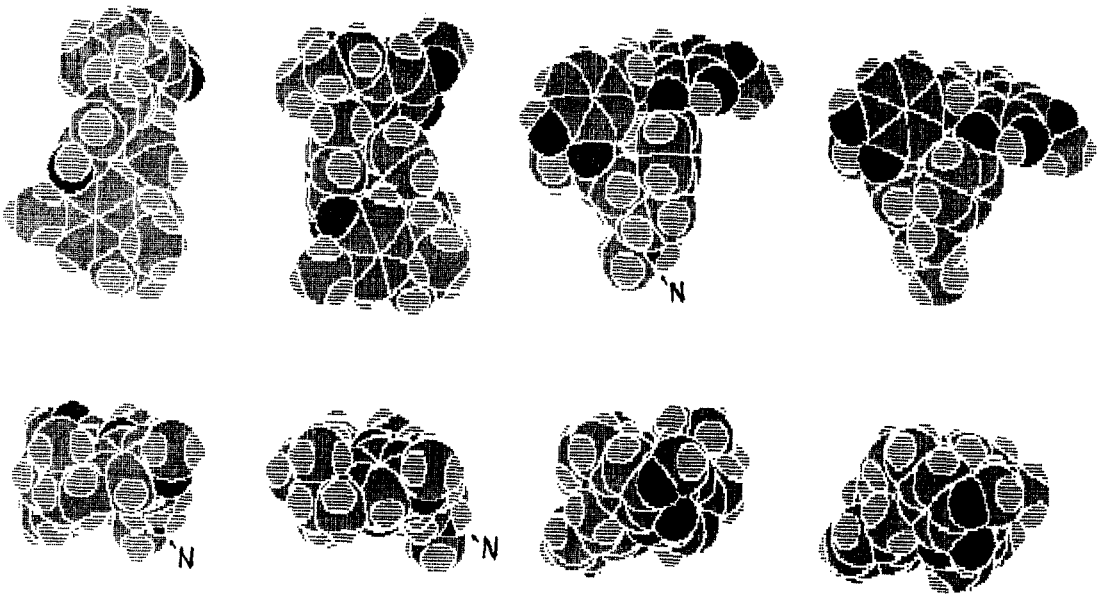

Fig. 3. Maximal-profile (top) and minimal-profile space-filling displays of the lowest minimum-energy conformations 1, 2, 3, and 4 (left to right) of d-tubocurarine. Carbon, oxygen, and hydrogen atoms are outlined in gray, black and hatched patterns, respectively. Nitrogen atoms (small black patterns labeled by *N*) are shielded almost completely by methyl groups.

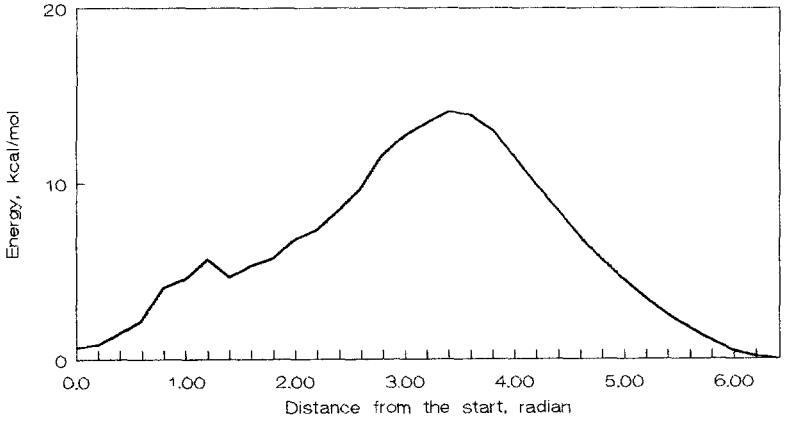

Fig. 4. Energy profile along the path from conformer 1 (start) to conformer 4 of the TC model with eliminated methoxy and hydroxy groups and atomic charges.

agreement between the three sets of experimental torsions of conformation 1 (RMSD = 6, 8 and 10°) is not better than that between experimental and calculated torsions. This shows the high reliability of the molecular mechanical calculations of TC.

Low-energy conformers 2 and 4, which were not detected experimentally, may also be responsible for the biological effects of TC. In particular, N–N separation in TC varies from 7.2 Å in conformer 4 to 11.3 Å in conformer 2 (Table 1), indicating that TC may bind to receptors of significantly different topographies.

To estimate the flexibility of TC, we calculated the energy profiles along the six paths of conformational inversion between the four lowest MEC. In doing so, we eliminated hydroxy and methoxy groups and atomic charges. Each profile was calculated in two opposite directions, their symmetry indicating that no bifurcation of the path was encountered. In the vicinity of each minimum, energy profiles were smooth sloping lines (*see*, for example, profile 1–4 in Fig. 4). The RMSD of torsional angles from the minima may be up to tens of degrees, with the energy increasing by less than 2 kcal/mol. In four of the six paths, the energies of the transition states (Table 3) did not exceed 19 kcal/mol. Therefore, the corresponding conformers easily transform into each other at room temperature. Two paths, 2–3 and 2–4, showed high energies for their transition states (24.6 and 28.4 kcal/mol, respectively),

Table 3. Characteristics of the paths of conformational inversions between the four lowest minimum-energy conformers of d-tubocurarine

Number of conformer on the path		Shortest distance between the conformers (radian)	Driven torsion	Transition state	
Start	Finish			Distance from the start (radian)	Energy (kcal/mol)
2	1	3.1	O11–C12	1.4	14.9
2	3	4.4	C28–O29	2.6	24.6
2	4	5.4	C28–O29	3.0	28.4
1	3	5.8	C33–C36	2.8	18.1
1	4	6.4	C28–O29	3.4	14.1
3	4	3.2	N1–C2	1.8	13.1

the corresponding transition times being from hours to days. However, indirect paths between conformers 2–3 and 2–4 exist. Thus, all the four lowest MEC of TC can transform into each other at room temperature indicating that TC is not a rigid molecule, as Pauling and Petcher (1973) have suggested.

Minimal Profile Dimensions of d-Tubocurarine

The minimal profile dimensions of the lowest MEC 1, 2, 3, and 4 were found to be 8.7 × 11.2, 8.8 × 12.3, 9.4 × 12.5, and 9.8 × 14.0 Å, respectively. Hence, the minimal dimensions of the parallelpiped-like channel which could accommodate TC correspond to the dimensions of conformer 1 (8.7 × 11.2 Å).

Conformations 1 and 2 have stretched overall shapes with the cationic heads most separated from each other; the axes passing through nitrogen atoms are roughly normal to the planes of minimal-profile projection. The smaller head with the mono methyl-substituted atom N1 may penetrate deeper into the channel with the tilted walls. We have estimated the minimal profile dimensions of the smaller head in conformers 1 and 2, adjusting them so that they would fit into the rectangular cavities of different depths (*see* Fig. 5). The minimal profile of the 9 Å depth cavity for conformer 2 is equal to 8.4 × 11.0 Å, whereas that of the 6 Å depth cavity is as small as 6.9 × 11.0 Å. For conformer 1, the minimal profile of the smaller head is equal to 8.9 × 11.1 Å in the cavity of 9 Å depth, and 6.9 × 11.1 Å in the cavity of 6 Å depth.

A Possible Model for d-Tubocurarine Binding within the Ion Channel

Recent models of the ion channel of AChR suggest a tapered shape of the pore with a wide entrance facing the extracellular space, and a more deeply located narrow part (Furois-Corbin & Pullman, 1989a; Charnet et al., 1990). In both conformers 1 and 2 of TC, the profile of the middle "biphenyl" segment is greater than that of the smaller cationic head, thus providing their binding to the channel with tilted inner walls.

Selyanko et al. (1988) suggested that TC competes with polymethylenebisammonium blockers (PMB) for a binding site which includes those regions of the neuronal AChR that normally interact with the penetrating cations. The dimensions of the tapered channel at the level of the more deeply located trialkylammonium head of PMB Et_3N–$(CH_2)_5$–$N(Alk)_3$ were recently deduced to be 6.1 × 8.3 Å (Zhorov et al., 1991). The minimal profile of one tetrahydroisoquinoline head of TC (6.9 × 11.0 Å, *see* Fig. 5*a*) is essentially greater. The lower limits for the channel dimensions at the level of the triethylammonium head of PMB were determined according to the profiles of the head in the three lowest energy conformers (5.7 × 9.1, 7.1 × 8.1, and 7.1 × 8.2 Å). Hence, although the two cationic heads of TC cannot bind simultaneously to both the nucleophilic regions in the binding site of PMB, TC and PMB may share one of the three successive nucleophilic regions within the channel.

Figure 6 presents the proposed model of the ion channel of neuronal AChR. The pore dimensions at the levels of nucleophilic regions 1 and 2 correspond to the model suggested earlier (Zhorov et al., 1991). Conformer 2 of TC interacts with regions 2 and 3, whereas the lowest energy conformer of PMB Et_3N–$(CH_2)_5$–$NMeEt_2$ interacts with regions 1 and 2. Although a hydrogen atom of TC penetrates the wall at the level of region 2 (*see* Fig. 6*b*), it will easily find room between the side chain groups in the real channel. A wider channel would be needed to fit the crystallographic conformer 1 of TC which is 0.5 Å wider in the critical dimension than con-

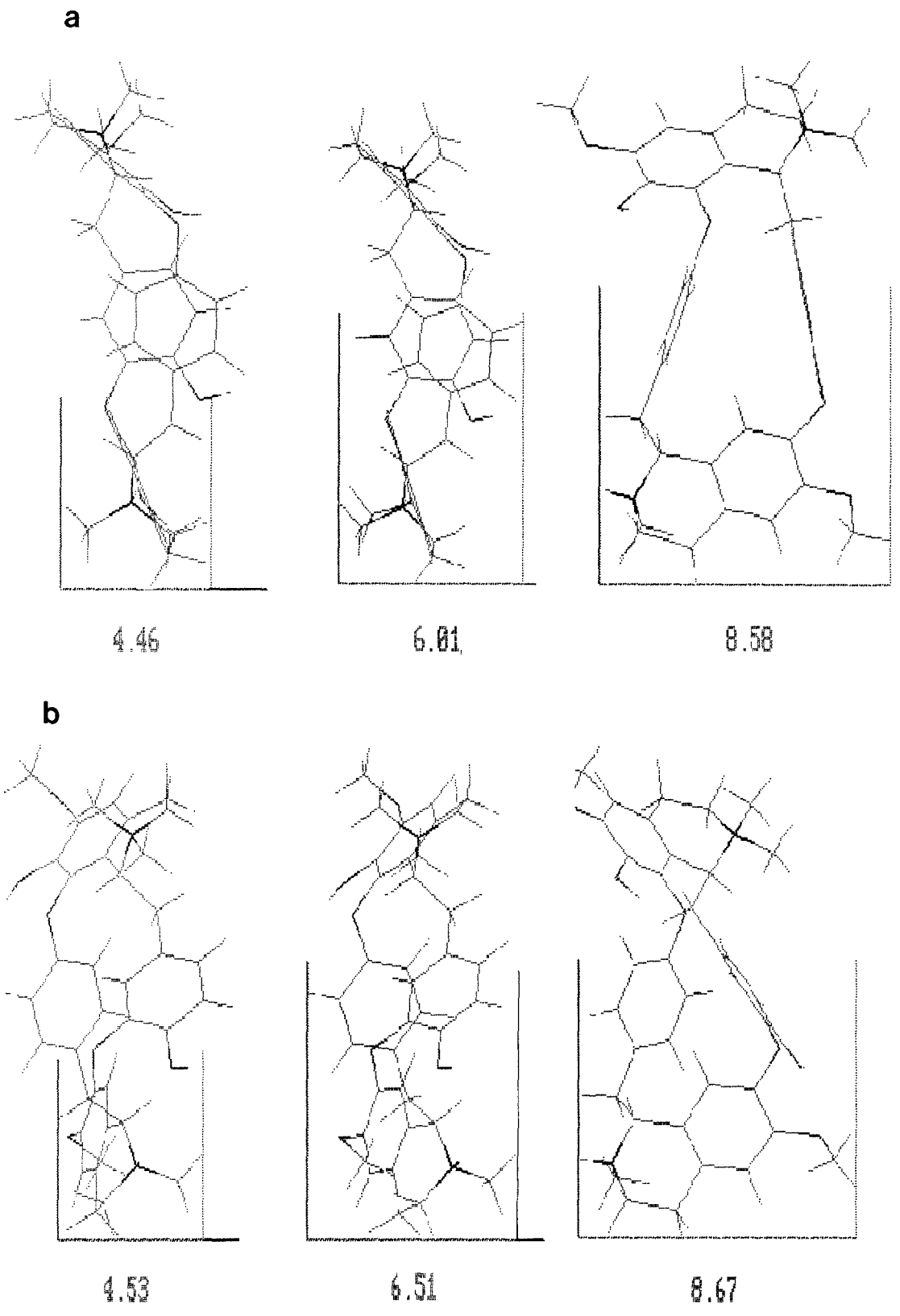

Fig. 5. Wire-frame displays of conformers 2 (*a*) and 1 (*b*) of d-tubocurarine fitting rectangular minimal-width cavities of 6 Å depth (left), 9 Å depth (middle), and a maximal-width cavity of 9 Å depth (right). Nitrogen and oxygen atoms are outlined with darker patterns than carbon and hydrogen atoms. Numbers displayed are the cavity widths (Å) fitting wire-frame models; the cavity width for the space-filling model is 2.4 Å greater. Note the slightly different scales of the displays.

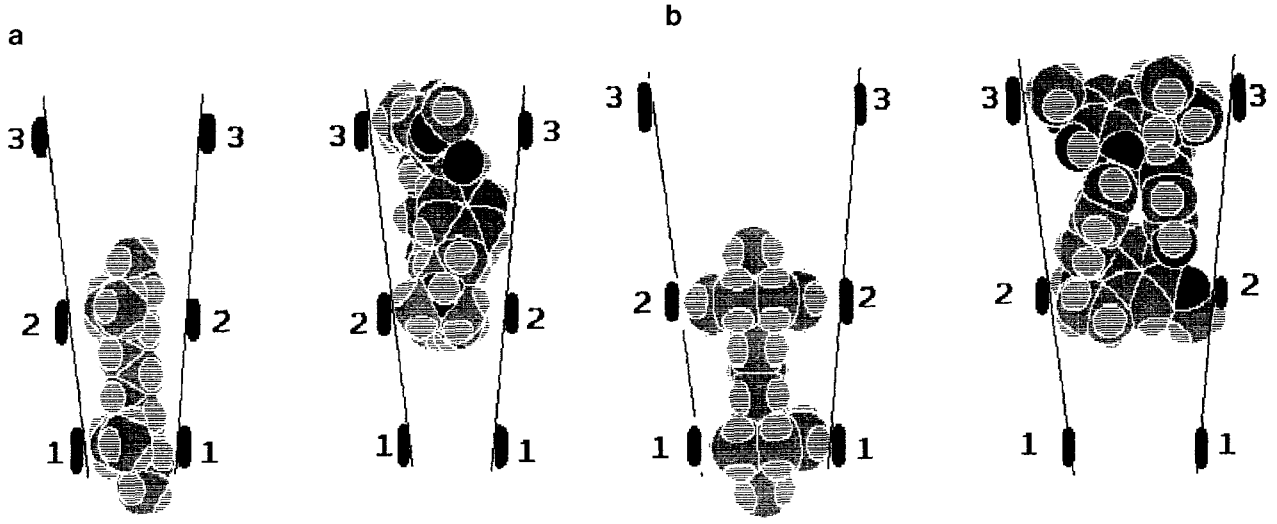

Fig. 6. Minimum-width (*a*) and maximum-width (*b*) space-filling displays of d-tubocurarine in conformation 2 (right) and of the compound Et_3N-$(CH_2)_5$-NEt_2Me in the most preferable conformation bound in the proposed model of the ion channel of neuronal acetylcholine receptor. The inner walls of the pore are presented as straight lines tilted to each other by 15°. *1*, *2* and *3* represent the nucleophilic regions of the channel.

former 2 (*cf.* Fig. 5*a* and *b*). The fact that the minimal-profile conformer 2 has been revealed in the calculations indicates that the crystallographic conformations of TC do not necessarily coincide with the biologically active conformations.

The nucleophilic regions 1, 2 and 3 in Fig. 6 may be related to the wide minima in the energy profile of Na^+ in the five MII-helix conical bundle modeling the ion channel of AChR (Furois-Corbin & Pullman, 1989*b*). Further experiments and calculations, in particular the docking of blockers into molecular models of the channel, are necessary to clarify the chemical nature of these regions.

The tilt of 15° between the walls of the pore agrees with the model of Furois-Corbin and Pullman (1989*a*). This agreement deserves attention because the two models are based on such different data as the amino acid sequence of the putative inner walls of the channel and the dimensions of the channel blockers.

In conclusion, the molecular mechanical calculations of TC reported here reproduce the experimentally observed conformations of the drug, predict new conformations, and demonstrate the conformational flexibility of the molecule. The suggested model of the ion channel of neuronal AChR explains the channel-blocking activity of TC and PMB and may be useful in the design of new channel blockers.

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