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Comparison of Lowest Energy Conformations of Dimethylcurine and Methoxyverapamil: Evidence of Ternary Association of Calcium Channel, Ca^{2+}, and Calcium Entry Blockers

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Abstract. Verapamil and dimethylcurine are Ca^{2+} entry blockers of essentially different chemical structures which presumably bind to the same arylalkylamine receptor of the L-type Ca channel. A systematic conformational analysis of methoxyverapamil (D-600) and dimethylcurine has been carried out using a molecular mechanics method. The lowest minimum-energy conformations of D-600 are predisposed to chelate Ca^{2+} by four oxygen atoms of the stacked methoxyphenyl moieties. Comparison of the lowest energy conformations of D-600-Ca^{2+} and dimethylcurine revealed a similar spatial disposition of cationic groups and methoxyphenyl moieties in the two compounds. A three-dimensional model of arylalkylamine receptor was suggested which incorporates two nucleophilic areas of the Ca channel. Dimethylcurine binds to these areas by its quaternary amine functions, whereas D-600 does so by amine function and via coordinated Ca^{2+}. The results support the hypotheses on ternary complex formation between the ligands of Ca channel, their receptors, and Ca^{2+}.

Key words: Ca^{2+} entry blockers — Conformation — Ca channel — Mechanism of blockade — Arylalkylamine receptor — Verapamil

Introduction

A challenging problem in molecular physiology is the detection of common mechanisms in the interaction of different membrane receptors with corresponding ligands. One of these mechanisms may be the ternary association of ligands, receptors, and Ca^{2+}. This hypothesis was proposed in our earlier studies which proved biologically active conformations of some adrenergic compounds to be predisposed for polydentate binding of metal ions (Zhorov & Govyrin, 1983). By analogy with adrenergic compounds, such Ca^{2+} entry blockers as verapamil and its methoxy derivative D-600 (*see* Fig. 1) were also implied to form ternary complexes with Ca^{2+} and Ca-binding groups within Ca channels (Zhorov & Govyrin, 1983, 1985*a*). Recently, Ananthanarayanan (1991*a*, *b*) presented experimental data that verapamil and other Ca antagonists bind Ca^{2+} and translocate it across the lipid bilayer, and suggested a similar hypothesis on ternary association of neurotransmitters, hormones, and drugs with Ca^{2+} and corresponding receptors.

The proposal on D-600-Ca^{2+}-receptor ternary association could be examined by the synthesis and subsequent demonstration of Ca^{2+} entry blocking activity of macrocyclic analogues of D-600 with methoxyphenyl fragments linked, in addition to the seven-membered chain, by a second chain incorporating quaternary amine function simulating chelated Ca^{2+}. Interestingly, curare alkaloids d-tubocurarine and curine have structural features which, to some extent, resemble required macrocyclic analogues of D-600 (*see* Fig. 1). Tubocurarine is known to block different types of ion channels (Manalis, 1977; Katz & Miledi, 1978; Ascher, Large & Rang, 1979; Colquhoun, Dreyer & Sheridan, 1979; Rang, 1982; Nohmi & Kuba, 1984). Dimethylcurine presumably blocks L-type Ca channels at the verapamil binding site (King et al., 1988).

These facts could be interpreted in terms of the

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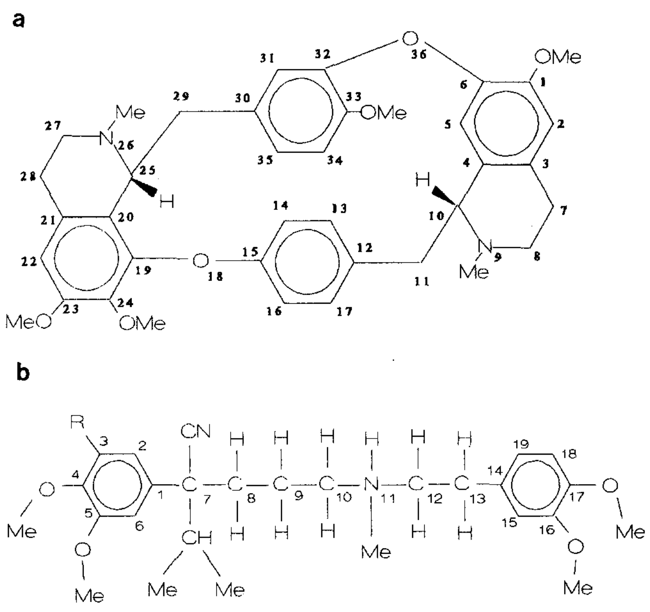

Fig. 1. Structural formulae of dimethylcurine (*a*) and D-600 (*b*) with numbered atoms in main chains of the compounds.

ternary complex hypothesis if supported by the data on similarity of the lowest energy conformations of dimethylcurine and D-600-Ca^{2+} complexes. In the present work, systematic conformational analysis of dimethylcurine, D-600, and D-600-Ca^{2+} has been carried out, and three-dimensional models of complexes of lowest energy conformations of dimethylcurine and D-600-Ca^{2+} with Ca channel are suggested. The models show a striking similarity in the spatial disposition of functional moieties of the two ligands interacting with the inner channel walls.

Materials and Methods

METHOD OF CALCULATIONS

Conformational analysis was carried out with an IBM PC/AT computer and Motorola 68020 workstation using the ZMM package, the last version of a universal program for molecular mechanisms calculations (Zhorov, 1975). This program minimizes conformational energy in the space of torsional and bond angles, the energy gradient being calculated by the analytical vector method (Zhorov, 1981, 1982). The energy was minimized by the Davidon method (Fletcher & Powell, 1963) until the RMS gradient became less than 0.1 kcal mol^{-1} rad^{-1}.

Nonbonded and electrostatic interactions, torsional energy, the energy of bond angles deformation and loop closing potential were taken into account. Nonbonded interactions were calculated with the potentials of Dashevskiy (1974). Electrostatic interactions were calculated with CNDO/2 charges and constant (ε = 4) or distance-dependent ($\varepsilon = R_{ij}$) dielectrics (Weiner et al., 1984). Bond lengths, fixed bond angles, parameters for flexible bond angles and for H-bond C≡N-HN in D-600 were chosen as in the work by Zhorov and Govyrin (1979). The torsional energy was calculated to be E_{tor} = (U/2) (1 − cos 2τ) for the bond Ph-O, and E_{tor} = (U/2) (1 + cos 3τ) for other bonds. The following barrier heights U (kcal/mol) were used: C_{sp3}-C_{sp3}, 3.0; C_{sp3}-C_{sp2}, 0; N-C, 2.0; N^+-C, 3.0; O-C_{sp3}, 1.0; O-Ph, 4.0. Recent calculations with this force field have proven the lowest energy conformations of d-tubocurarine to be in good agreement with the X-ray data (Zhorov & Brovtsyna, 1993).

The generation of starting points for energy minimization of dimethylcurine was made by systematic and random procedures. A systematic generation of starting points with a closed 18-membered ring was made by the method of Go and Scheraga (1970). Random starting points with approximately closed main 18-membered ring and 6-membered rings of tetrahydroisoquinoline fragments were selected from many conformations with torsional angles delivered by the random number generator.

At the first stage of the calculations of dimethylcurine, the methoxy and N-methyl groups were replaced by the hydrogen atoms, and a model DCM1 with rigid bonds, atoms, and phenyl rings was analyzed. Model DCM2 with 16 flexible atoms (3, 4, 7–11, 18, 20, 21, 25–29, 36) in the most strained molecule fragments was used to refine the energy and geometry of the lowest energy conformations of DCM1. The model DCM3 has the methoxy and N-methyl groups, R-configuration of the quaternary nitrogen atoms, and the same flexible atoms as DCM2. Electrostatic interactions in DCM1–DCM3 were not taken into account because of indefinite atomic charges (in a random minimum-energy conformation of the macrocycle, overlapping atoms may have nonrealistic electron densities). Atomic charges were calculated by the CNDO/2 method for the lowest energy conformation of DCM3 and used in DCM4 which coincides with DCM3 by other parameters.

The computational model of protonated (7R, 11S)-D-600 incorporates 12 flexible atoms (7 atoms in the main chain, branched carbon of isopropyl group, and oxygen atoms). Atomic charges were calculated by the CNDO/2 method with the coordinates of atoms obtained by energy minimization of the fully extended conformation of D-600.

If during energy minimization a torsion deviated from its

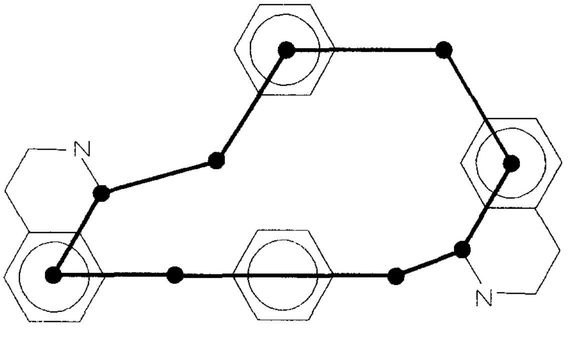

Fig. 2. 9-membered virtual cycle, a model for the 18-membered main ring of dimethylcurine.

starting value by more than 60°, the minimization was terminated and the corresponding conformation was neglected.

Results and Discussion

CONFORMATIONS OF DIMETHYLCURINE

The systematic search for the minimum-energy conformations of dimethylcurine 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 calculation of 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, all the loop-closing values of the dependent torsions in each knot of the $(n - 6)$-dimensional grid of independent torsions had to be calculated. The main cycle of dimethylcurine incorporates 18 bonds which make 12 independent torsions. Eight of these torsions are within the rigid aromatic cycles and have definite values (0 or 180°). Two bonds in the para-position of the phenyl ring, C11-C12 and C15-O18, make one line, and the two corresponding torsions can be substituted by a single one which specifies the rotation of the fragment C11-Ph-O18 around the virtual bond C11-O18. This leaves only three independent torsions.

Figure 2 shows a 9-membered virtual cycle which was constructed as an approximation to the main cycle of dimethylcurine. Three independent torsions of the cycle were varied with 30° spacing to give a grid of 3^{12} knots. In each knot of the grid, the procedure by Go and Scheraga (1970) was carried out. Among main ring closed conformations only those were filtered which provided a closure of the saturated rings in tetrahydroisoquinoline fragments. This procedure yielded 377 starting points. Energy minimization from them delivered 51 minimum-energy conformations of DCM1 with energy <120 kcal/mol, including 9 conformations with energy <12 kcal/mol. To test if some low-energy conformers were missed during a systematic search, 59 random starting conformers of DCM1 were generated. Energy minimization from them reproduced four of the nine lowest conformers found by the systematic search, yielding no new conformers with energy <12 kcal/mol.

The energy and geometry of the nine lowest minimum-energy conformations of DCM1 were refined by using DCM2 with 16 flexible atoms in the most strained fragments of molecule. These refined conformations were then combined with 32 possible orientations of methoxy groups (4 torsions C-C-O-Me being equal to 90 or −90°) to give 288 starting points for energy minimization of DCM3. According to these calculations, introduction of N-methyl and methoxy groups did not shift significantly conformational equilibrium of DCM2, and most of the orientations of methoxy groups were possible for each conformation of its main loop. Table 1 presents the nine lowest-energy conformations of DCM3. Wire-frame displays of conformers 1–8 are shown in Fig. 3.

Electrostatic interactions were taken into account in energy minimization of DCM4 from the nine starting points given in Table 1. Most of the conformers in DCM4 calculated with $\varepsilon = 4$ differ from their counterparts in DCM3 by less than 5° in torsional angles, and by less than 1.2 kcal/mol in relative energy (*see* Table 1). Only conformers 6 and 9 of DCM4 have high energy due to repulsion between closely spaced ammonium groups. Similar results were obtained for DCM4 calculated with $\varepsilon = R_{ij}$.

CONFORMATIONS OF D-600

The search for the lowest minimum-energy conformations of the protonated D-600 was performed in two stages. In the first stage, a diminished model of D-600 with eliminated methoxy fragments and neglected electrostatic interactions was investigated. Starting torsions were sampled as 2187 (3^7) combinations of t-, g-, and g$^-$ -conformations of the bonds C7-C8, C8-C9, C9-C10, C10-N11, N11-C12, C12-C13, and C7-^{i}Pr with τ(C-Ph) = 90°, τ(C-CH$_3$) = τ(N-CH$_3$) = 60°. Energy minimization at this stage gave 616 conformers with energy <20 kcal/mol; a list of 39 conformers with energy <3 kcal/mol is given in Table 2.

In the second stage, 60 minimum-energy conformations of the diminished model of D-600 with energy <3.5 kcal/mol were combined with 64 orienta-

Table 1. Energy (kcal/mol), torsional angles (degree), minimal-profile dimensions a × b (Å), and N,N distance (Å) of optimal conformers[a] of dimethylcurine

Number	1	2	3	4	5	6	7	8	9
Energy (DCM3)	0.0	0.9	0.9	2.3	3.3	3.4	3.5	3.9	6.9
Energy (DCM4, $\varepsilon = 4$)	1.2	1.2	0.0	1.2	2.3	6.3	3.6	3.3	13.6
Energy (DCM4, $\varepsilon = R$)	1.4	1.2	0.0	1.5	2.4	6.9	3.7	3.1	10.3
Torsions (DCM3)									
5- 4-10-11	84	78	85	88	44	76	75	40	51
4-10-11-12	-66	-59	-38	-54	-74	-74	-63	21	-82
10-11-12-13	-79	-62	-69	95	126	-92	90	55	-71
14-15-18-19	3	-26	-147	43	37	17	-52	31	22
15-18-19-20	77	97	-119	-126	-122	61	-93	-124	58
19-20-25-29	64	46	79	73	79	75	88	75	75
20-25-29-30	-125	-108	-142	-99	-98	-168	36	-132	-178
5- 6-36-32	-62	-65	-122	-49	29	39	37	-121	66
6-36-32-31	114	124	49	-80	-130	-98	-86	29	-90
4-10- 9- 8	-58	-54	-57	-60	41	-53	-51	43	34
10- 9- 8- 7	58	60	56	54	-65	62	61	-65	-63
4- 3- 7- 8	-1	5	-2	-7	-23	8	9	-21	-28
20-25-26-27	-45	40	-55	-53	-57	-59	-64	-51	-59
25-26-27-28	62	-66	59	63	61	62	51	60	62
20-21-28-27	17	-25	4	10	4	4	-12	8	5
24-23-O-Me	121	123	115	117	112	-81	-81	115	116
19-24-O-Me	97	104	81	81	82	89	83	82	86
32-33-O-Me	115	117	120	-133	-133	-109	-105	78	77
2- 1-O-Me	-100	-100	59	76	-100	-67	-63	51	-54
Dimensions (DCM3)									
a	8.0	7.1	5.7	8.7	5.8	8.0	8.6	8.0	9.0
b	8.7	9.1	11.3	8.7	10.2	8.4	9.0	11.7	9.1
N,N	8.3	9.6	11.0	11.4	11.4	7.1	9.9	10.3	6.9

[a] Each conformer is the lowest energy one in a group with similar main chain and different orientation of methoxy moieties.

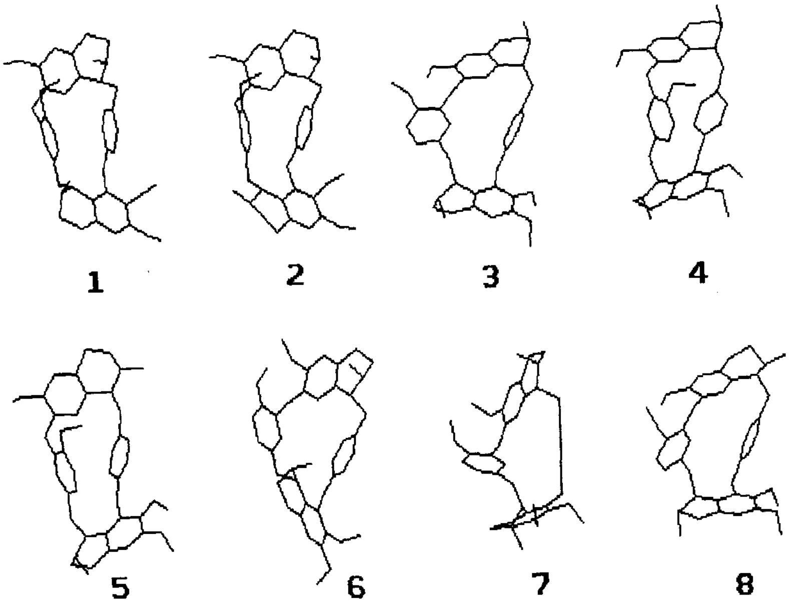

Fig. 3. Wireframe display of the lowest minimum-energy conformations 1-8 of dimethylcurine (model 3). Conformers are numbered as in Table 1. Hydrogen atoms are omitted.

Table 2. Designation[a] and energy (kcal/mol) of the lowest minimum-energy conformations of D-600 diminished model

$t\ g\ g^-t\ g^-g^{-c}$	0.0	$g^-g^-t\ g^-g^-t^{\ b}$	1.5	$g^-g^+t\ g^-g^-g^-$	2.5
$g^-g^-t\ g^-g^-t$	0.0	$g^-t\ g^-g^-g^-t^{\ b}$	1.6	$g^-g^-t\ g^+g^+g^-$	2.5
$g^-g^-g^-t\ t\ g^+$	0.1	$g^-t\ g^-t\ t\ g^+$	1.6	$g^-t\ t\ g^-g^-g^+$	2.6
$g^+t\ g^+g^+g^+t$	0.7	$g^-g^+t\ g^+g^+g^{+c}$	1.8	$g^+g^+t\ g^+g^+t$	2.6
$g^-t\ g^-g^-g^-t$	0.7	$g^-t\ t\ g^+g^+g^-$	2.0	$g^-t\ t\ g^-g^-g^+$	2.6
$g^-g^-g^-t\ g^-g^+$	0.8	$g^+t\ g^+g^+g^+g^-$	2.1	$g^-g^-t\ g^+t\ g^+$	2.6
$g^+t\ g^+g^+t\ g^+$	0.9	$g^-g^-t\ t\ g^+g^+$	2.2	$g^-t\ g^-g^-t\ t$	2.6
$g^-t\ g^+g^+t\ g^+$	1.0	$g^-t\ g^-g^-g^-g^+$	2.2	$g^-t\ g^-g^+g^+t$	2.7
$g^-g^-t\ g^-g^-g^-$	1.0	$g^-g^-g^-t\ g^-g^{+b}$	2.2	$g^-t\ g^-g^+t\ g^+$	2.8
$g^+t\ g^+g^+t\ t$	1.1	$g^-g^+t\ g^+g^+g^+$	2.3	$g^+g^+g^+g^+t\ g^+$	2.8
$g^-g^+t\ g^-g^-g^+$	1.2	$t\ t\ t\ g^+g^+g^{+c}$	2.3	$g^-g^-g^+g^+g^+t$	2.9
$g^+t\ t\ g^-g^-g^+$	1.4	$g^+t\ t\ g^-g^-t$	2.3	$g^-g^-g^+g^+t\ g^+$	3.0
$g^-g^-g^-t\ g^+g^-$	1.5	$g^+g^-g^-t\ t\ g^+$	2.4	$g^+t\ g^+g^-g^-t$	3.0

[a] Symbols in each group, left to right, correspond to torsions τ(1-7-8-9), τ(7-8-9-10), τ(8-9-10-11), τ(9-10-11-12), τ(10-11-12-13),and τ(11-12-13-14). Torsion, τ(1-7-30-31) is *trans* in conformers marked with [b], (−)-gauche in conformers marked with [c], and (+)-gauche in other conformers.

Table 3. Energy (kcal/mol) and main torsional angles (degrees) of the lowest energy conformations of D-600[a]

Number	1	2	3	4	5	6	7	8	9
Energy (ε =4)	0.0	0.1	0.4	0.5	0.6	0.8	0.9	1.0	1.0
Energy ($\varepsilon = R_{ij}$)	0 0	0.3	1.6	1.3	0.5	1.3	0.1	1.6	1.2
Torsions[b]									
2- 1- 7- 8	−48	134	75	−64	−33	111	−46	−82	114
1- 7- 8- 9	−46	−43	64	−38	187	65	−50	69	−59
7- 8- 9-10	−61	−50	−167	−57	102	166	−73	−180	142
8- 9-10-11	177	180	58	147	−83	172	79	61	−65
9-10-11-12	−54	−52	52	65	169	−61	49	68	164
10-11-12-13	−57	−56	58	−162	−64	−61	59	−166	−167
11-12-13-14	172	−66	−177	58	−49	79	−169	73	52
12-13-14-15	146	87	107	59	109	63	80	−79	44
2- 3-O-C	−63	65	−105	−42	58	−105	−58	57	−65
3- 4-O-C	−94	−78	99	−77	88	85	−85	84	−95
6- 5-O-C	59	69	−55	−76	52	67	−104	47	−105
15-16-O-C	59	60	51	−108	103	67	53	98	−93
16-17-O-C	84	86	−116	−36	220	−123	−71	−138	−121
1- 7-30-31	60	60	60	58	−61	61	60	59	60

[a] Each conformer is the lowest energy one in a group with similar main chain and different orientations of methoxy and dimethoxyphenyl moieties.

[b] Calculated with $\varepsilon = 4$. Torsions calculated with $\varepsilon = R_{ij}$ differ from those given in the table by less than 2°.

tions of methoxy and dimethoxyphenyl moieties (torsions C-C-O-Me and C13-C14 being equal to 90 or −90°) to give a total of 3840 starting points. Starting torsion of the bond C1-C7 was taken as 90°, of the bonds C-Me, N-Me, and O-Me as 60°. This stage gave 443 minimum-energy conformations of D-600 with energy <3 kcal/mol. Table 3 and Fig. 4 show the lowest minimum-energy conformations of D-600, each being optimal in the group with similar conformation of the main chain and different orientations of methoxy and dimethoxyphenyl moieties.

Conformational analysis of D-600 revealed essential preference of the folded conformations over extended ones due to nonbonded attraction between two aryl moieties; the energy of the fully extended minimum-energy conformation of D-600 is equal to 13.7 kcal/mol. This result disagrees with the data of Brasseur, Deleers and Malaisse (1983) which showed folded and extended conformations of D-600 to be in equilibrium. This disagreement follows evidently from the different methods of starting points treatment. Although Brasseur et al. (1983)

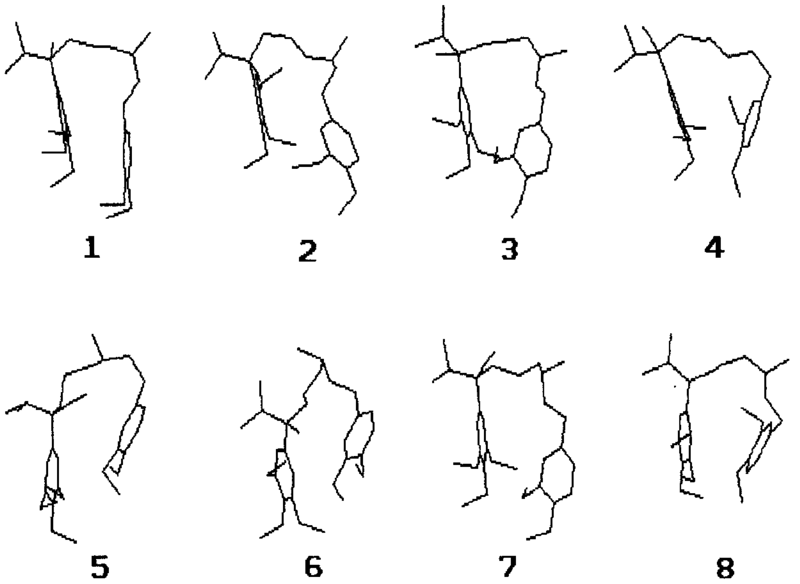

Fig. 4. Wireframe display of the lowest minimum-energy conformations 1-8 of D-600. Conformers are numbered as in Table 3. Hydrogen atoms are omitted.

generated many starting points, the energy minimization was fulfilled if starting energy fell within a cutoff level. Thus, many folded starting conformations could be neglected due to overlap of the van der Waal's spheres. At the same time, few iterations of energy minimization, as a rule, take off this overlap, providing a good approximation for further energy minimization.

D-600-Ca^{2+} COMPLEXES

Figure 4 shows that the lowest minimum-energy conformations of D-600 are predisposed to polydentate binding of metal ions by oxygen atoms of the spatially close dimethoxyphenyl and trimethoxyphenyl moieties. This agrees with the experimental data of Ananthanarayanan (1991*b*) that verapamil binds Ca^{2+} in acetonitrile and translocates Ca^{2+} across the lipid bilayer.

To estimate the geometry and relative energy of possible chelate complexes, minimum-energy conformations of D-600 predisposed to tetradentate binding of Ca^{2+} (conformers 1, 3–7) were used as starting points for energy minimization of D-600-Ca^{2+} complexes. Implementing an interactive molecular graphics program (DTMM), Ca^{2+} was positioned manually against four closely spaced oxygen atoms of D-600. Methyl moieties of methoxy groups were moved away from Ca^{2+} by changing Ph-O torsions. The ZMM package was then used to pick up the geometry of the displayed complex and to minimize energy in the space of torsional and bond angles of D-600 and Cartesian coordinates of Ca^{2+}.

In these calculations, an oxygen atom with charge $+2e_0$ was chosen as a model for Ca^{2+}. Although the crystallographic radius of Ca^{2+} (1 Å) is less than that of the oxygen atom (1.4 Å), in minimum-energy complexes, the electrostatic attraction and nonbonded repulsion between Ca^{2+} and oxygen atoms were counterbalanced, and the distances Ca^{2+}-O fell in the range 2.3–2.4 Å, which is close to the experimental values.

As could be expected, the results of these calculations depend strongly on the value of dielectric constant ε. Calculations with $\varepsilon = 4$ gave equilibrium complexes with energy 2–4 kcal/mol relative to the global minimum of the lone D-600. The complexes are meta-stable, for removing Ca^{2+} in infinity requires overcoming a barrier of about 3–4 kcal/mol. The complexes are energetically unfavorable due to electrostatic repulsion between Ca^{2+} and protonated amino groups. Within the Ca channel this repulsion may be compensated by attraction of Ca^{2+} and D-600 to the receptor, and by the presence of counter-ions. It is not excluded also that D-600 binds to the Ca channel in deprotonated form: uncharged analogues of verapamil were shown to inhibit myocardial contractility (Cohen et al., 1987).

Calculations with distance-dependent dielectric $\varepsilon = R_{ij}$ have shown the chelate complexes to be preferable, with the energy about -30 kcal/mol relative to the global minimum of the lone D-600 (*see* Table 4). This estimate, however, cannot be considered as a measure of stability of the complex: chelation of a metal ion by an organic ligand is a complex process with stages of ion and ligand dehydration, binding of ion to ligand, and complex hydra-

Table 4. Energy (kcal/mol), main torsional and bond angles (degree), minimal-profile dimensions a × b (Å), and N,Ca^{2+} separation (Å) of optimal conformations[a] of D-600-Ca^{2+} complex calculated with distance-dependent dielectric

Number[a]	5	1	6	3	7	4
Energy[b]	−33.9	−31.9	−31.2	−29.8	−28.5	−26.4
Torsions						
2- 1- 7- 8	−40	−40	99	69	−48	−83
1- 7- 8- 9	−156	−59	60	66	−56	−43
7- 8- 9-10	102	−70	173	−181	−83	−65
8- 9-10-11	−81	218	178	57	77	148
9-10-11-12	165	−59	−59	52	45	64
10-11-12-13	−69	−65	−62	61	59	−164
11-12-13-14	−61	98	95	−146	−146	58
12-13-14-15	135	237	76	96	94	39
2- 3-O-C	−52	−53	47	−104	−29	−41
3- 4-O-C	−125	−79	59	73	−106	−106
6- 5-O-C	53	42	41	−31	51	45
15-16-O-C	12	5	4	21	11	11
16-17-O-C	197	−187	187	−216	148	198
Bond angles						
1- 7- 8	108.7	111.3	107.8	108.0	111.1	107.6
7- 8- 9	120.1	118.7	115.8	115.7	119.0	117.4
8- 9-10	114.4	114.0	113.0	113.0	120.5	117.7
9-10-11	118.5	117.2	116.1	116.8	122.3	113.2
10-11-12	111.6	114.0	113.9	112.7	112.4	111.8
11-12-13	115.8	115.5	116.7	116.7	117.7	114.9
12-13-14	116.0	116.5	116.4	110.4	109.8	114.6
Dimensions						
a	7.0	7.5	7.4	8.4	7.9	8.1
b	8.6	9.5	8.8	8.2	8.0	8.6
N,Ca^{2+}	8.0	8.4	8.5	8.3	8.3	7.1

[a] Number of conformers of lone D-600 (*see* Table 3).

[b] Relative to the global minimum of lone D-600 calculated with distance-dependent dielectric.

tion. Although molecular mechanics calculations alone cannot predict the stability of the chelate complex, they show whether some minimum energy conformations are predisposed to chelation. The realization of this predisposition depends on the environment. The Ca channel may offer a good environment for the chelation.

MODELS OF DIMETHYLCURINE AND D-600-Ca^{2+} BINDING WITHIN THE Ca CHANNEL

Although no direct experimental evidence of arylalkylamine receptor location within the Ca channel is available, the data on potential-dependent (Porzing & Becker, 1988) and use-dependent (Tytgat, Vereecke & Carmeliet, 1988) action of verapamil may be interpreted in favor of such location. In this case, the profile of the channel at the receptor level may correspond to the minimal profile of the lowest energy conformers of the ligands (Zhorov et al., 1991).

Using a molecular graphics technique described elsewhere (Voitenco et al., 1991), the dimensions of rectangles that accommodate the minimal-profile projections of the optimal conformers of DCM3 and D-600-Ca^{2+} were estimated (*see* Tables 1, 4). A number of dimethylcurine and D-600-Ca^{2+} conformers have similar minimal-profile dimensions and distances between cationic groups, the axes passing through the groups being roughly normal to the planes of minimal-profile projections. For conformer 1 of dimethylcurine, the dimensions of the minimal-profile projection increased by 2.4 Å (doubled van der Waals radius of hydrogen atom) are equal to 10.4 × 11.1 Å, and for conformer 5 of D-600-Ca^{2+}, 9.4 × 11.0 Å. These values may reflect the dimensions of the Ca channel at the level of arylalkylamine receptor.

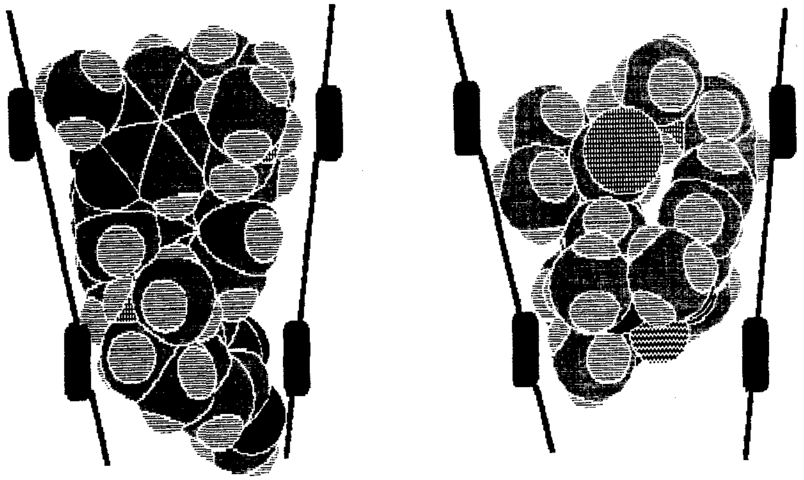

Fig. 5. Space-filling displays of dimethylcurine (conformer 1) and D-600-Ca^{2+} (conformer 5). The following patterns are used to outline atoms: carbon: gray; hydrogens: light gray, nitrogen: grid, oxygen: black, calcium: hatched. The channel walls are shown as straight lines with black spots indicating the level of nucleophilic areas.

A remarkable feature of the optimal conformations of dimethylcurine and D-600-Ca^{2+} is their tapered shape (*see* Fig. 5). This feature is characteristic for pentamethylen-bisammonium compounds (Zhorov et al., 1991), d-tubocurarine (Zhorov & Brovtsyna, 1993), chlorpromazine (Furois-Corbin & Pullman, 1989), and probably for many other blockers of ionic channels. An obvious consequence of this feature is the ability of the blockers to fit ionic channels with the tapered walls. As this takes place, the sharp end of the blocker should get deeper in the channel than the wider end.

Figure 5 presents the proposed models of the L-type Ca channel with two nucleophilic areas at the tapered straight inner walls and bound lowest energy conformations of dimethylcurine and D-600-Ca^{2+}. The models show a similar disposition of charged groups and phenyl rings in the two ligands, explaining that such different compounds as D-600 and dimethylcurine bind to the same arylalkylamine receptor (King et al., 1988).

The suggested model attempts to speculate on the sequence of association of D-600, Ca^{2+}, and Ca channel. There are three possibilities for this process: (1) Ca^{2+} binds to channel-D-600; (2) D-600 binds to channel-Ca^{2+}; (3) D-600-Ca^{2+} binds to the channel. The fact that Ca^{2+} forms the sharp end of the D-600-Ca^{2+} complex makes model (1) improbable, for Ca^{2+} should approach D-600 either from the extracellular (outer) mouth of the channel already filled by D-600, or by moving against the membrane potential from the inner mouth. Model (2) is also doubtful due to the speedy passing of Ca^{2+} along the Ca-binding group in the open channel; besides, D-600 is unlikely to move in the field of membrane potential with neutral methoxy functions forward and protonated amino function backward.

The most probable seems model (3) where membrane potential drives Ca^{2+}-D-600 into the channel. This model agrees with the ability of verapamil to translocate Ca^{2+} through the lipid bilayer (Ananthanaryayanan, 1991*b*). In this case, D-600 serves as an intrareceptor ionophore, the property first suggested for the ligands of adrenergic receptors and Ca channels (Zhorov & Govyrin, 1983, 1985*a*), and which may be inherent for many neurotransmitters, drugs and hormones (Zhorov & Govyrin, 1985*b*; Ananthanarayanan, 1991*a*).

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