A Receptor Interaction Model for Phenylcarbamate Local Anesthetics

V. Kettmann and J. Sivý

Department of Analytical Chemistry, Faculty of Pharmacy, Comenius University,
Odbojárov 10, 83232 Bratislava, Slovak Republic

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We report here a molecular modeling study of selected conformationally constrained phenylcarbamate local anesthetics in relation to the available pharmacological data that enabled us to develop a receptor-interaction model for this class of drugs. The validity of the model was confirmed on other semirigid analogues prepared for this study. The results suggest that the phenyl ring is most likely involved in a stacking interaction with a complementary receptor site and the tertiary ammonium group is capable of both hydrogen bonding and lipophilic interactions.

Introduction

This work is part of a project aiming at an understanding of some of the structural and physicochemical determinants of local anesthetic and related (e.g. antiarrhythmic) activity of phenylcarbamate (pentacaine) derivatives which are esters of alkoxyphenylcarbamoic acid with aminoalcohols (Beneš *et al.*, 1972). Common structural features of the active compounds of this type are a phenylcarbamate function, a tertiary amine, and hydrophobic groups attached to the phenyl ring and the amine nitrogen. The phenylcarbamate and the amine moieties, which are connected via a linking chain (this typically consists of 2 or 3 carbon atoms), are assumed to participate directly in the drug-receptor interaction and hence constitutes two major pharmacophores (Remko and Scheiner, 1988; Gregáň and Polášek, 1992; Gregáň *et al.*, 1993a).

Previous results from this and other laboratories have shown that the *in vivo* and *in vitro* activity of these compounds is dependent upon the molecular lipophilicity (as modeled by log P and related parameters) (Gregáň and Polášek, 1992; Gregáň *et al.*, 1993b)as well as upon the spatial distribution of the two pharmacophores (Gregáň *et al.*, 1993b; Beneš *et al.*, 1982). As expected for a passive transport process, we found a parabolic relationship between the *in vivo* activity and lipophilicity, with the maximum activity lying in the interval $(\log P)_{\text{opt}} \simeq 3.5 - 4.0$ (Gregáň *et al.*, 1993b). Such a pharmacological behaviour, which is typical for drugs acting at specific receptor sites located either within the phospholipid membrane domain or on the intracellular surface of the membrane, has also been observed for other structural classes of local anesthetics and related drugs (Mason *et al.*, 1991). Since it is well established, from both electrophysiological and binding studies (Hille, 1984; Sheldon *et al.*, 1991), that the latter compounds (e.g. lidocaine, a prototypic class Ib agent) bind specifically on the intracellular face of the voltage-operated sodium channel, it is very likely that also the phenylcarbamate class local anesthetics act as competitive and reversible ligands at similar, if not identical, binding sites.

In an attempt to give better insight into the spatial requirements of the local anesthetic activity, which are still poorly understood, we prepared (Gregáň *et al.*, 1994) two pairs of *cis* and *trans* isomers **1**-**4** (Fig. 1), the conformational flexibility of which was restricted by embedding two atoms of the 3-carbon linker into a seven-membered ring system; the crystal structures of the 2′-butoxy pair of isomers were subsequently determined by an X-ray analysis (Kettmann *et al.*, 1993a; Kettmann *et al.*, 1993b). However, preliminary *in vivo* tests surprisingly showed the two isomers to be nearly equiactive and this unexpected result could not be adequately explained in terms of the crystallographic studies alone. Consequently, we initiated molecular mechanics and molecular modeling studies on **1**-**4** and, to have a reference frame

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Reprint requests to Dr. Kettmann.

for the molecular comparisons, all four diastereomers **5-8** (Fig. 1) possible for another semirigid derivative of this chemical class for which the biological data were reported previously (Beneš *et al.*, 1982) have also been included in the analysis. At the same time, the latter compounds offered us an opportunity to develop a receptor-interaction model for this type of drugs, which is believed to be of help in future designing of new drugs.

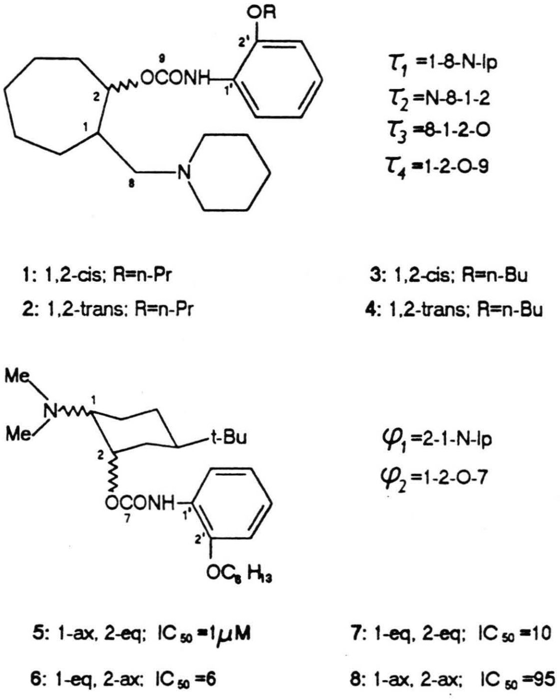

Fig. 1. Chemical structures of the compounds pertinent to this study. τ_1-τ_4 are biologically relevant torsion angles at the rotatable bonds in compounds **1-4** (lp means lone pair of electrons on the amino nitrogen); φ_1 and φ_2 are similar variables for compounds **5-8**. The orientation of the substituents on the cyclohexane ring in compounds **5-8** is denoted by ax (axial) or eq (equatorial). The IC_{50} values for compounds **5-8** were measured *in vitro* by inhibiting nerve-elicited guinea-pig ileum twitches as described by Beneš *et al.* (1982).

Experimental

Pharmacology

in vivo activity of compounds **1-4** in surface anesthesia (rabbit cornea, 10 mM cocaine as standard) and infiltration anesthesia (intradermal application to guinea pigs, 20 mM procaine as standard) was determined as the relative potency of the drugs with respect to standards using a graphic technique described in detail by Vrba and Sekera (1959). In short, for both types of anesthesia, 5 different concentrations (which caused anesthesia lasting in the range 10-40 min) of the drugs were used and reflexive irritation was measured using metal estesiometer in 3-min. intervals after the local anesthetic application. After graphic extrapolation the equieffective concentration of the compounds tested were found and the relative activity was expressed as a ratio of the equieffective concentrations of standard and the compound.

Molecular modeling

Studies on molecular comparisons and superimpositions were performed on model compounds **1a**, **2a**, **5a-8a**, which differ from the corresponding compounds **1-8** in having a 2'-methoxy group on the phenyl ring and, in addition, the *tert*-butyl group of compounds **5-8** was replaced by an H atom. The molecular modeling program MOLGEN (Baričič and Mackov, 1990) was used.

The rationale for the selection of the diastereomers **5a-8a** for model development was the following: (a) the pharmacological data, based on electrophysiological studies measuring depression of action potential amplitude of isolated rat sciatic nerve and concentration-dependent effect on the nerve-elicited twitches of guinea pig ileum (Beneš *et al.*, 1982),are most accurate among the phenylcarbamate local anesthetics; (b) the potency of the individual isomers is well separated, covering a range of almost 2 orders of magnitude; (c) the isomers are isolipophilic so that, despite the use of the *in vitro* data, any variations in activity must reflect differences in the drug receptor-interaction; and (d) these compounds are most rigid among this type of drugs.

The general strategy adopted in the modeling studies was the following: (a) the phenylcarbamate portion of all structures was superposed, the fitting points being the four atoms of the carbamate moiety and the C1' and C4' atoms of the phenyl ring; the quality of the superimpositions was evaluated by the root mean square (rms) deviation between the fit atoms; (b) assuming that the protonated amine forms a hydrogen bond with an anionic site (e.g. O^- of a carboxylate residue) of the receptor, we introduced a dummy atom (A_i) located 2.8 Å from the amine nitrogen and aligned with its lone electron pair (N^+–H bond); and (c) conformations at the rotatable bonds were varied systematically to minimize the $|A_{opt} - A_i| = \Delta A_i$ distance, where A_{opt} corresponds to the most active isomer **5a** in its receptor-bound conformation (see below) which was used as a template in these superimpositions; we considered the conformers to be capable of hydrogen bonding to the presumed anionic site if $\Delta A_{fit} \leq 0.3$ Å and if the cost in internal energy to attain the fit conformation was within 13 kJ/mol of the global minimum.

Conformational (steric) energy (and geometry) was calculated by using the molecular mechanics program MMP2(85) (Allinger, 1985) and parameter set. Although these compounds are known to be protonated when interacting with the receptor, due to a lack of reliable parameters for a protonated amine, all calculations were carried out on the free bases; however, an excellent agreement between the protonated and unprotonated forms has been observed previously, with the differences in energy being at most 4.2 kJ/mol (Froimowitz and Rämsby, 1991).Input coordinates for **1a** and **2a** were obtained from the X-ray structure of **3** and **4**, respectively. Due to the lack of X-ray crystal coordinates for the selected compounds **5a**-**8a**, their initial geometry was generated by using the BUILD option within MOLGEN. Since none of the racemic compounds of this type was resolved into its enantiomers, chirality of the reference compound **5a** was arbitrarily chosen as 1-*S*, 2-*R*; then it was found during the super-imposition studies that the remaining compounds conform to the model with the following stereochemistry: **6a**, 1-*S*, 2-*R*; **7a**, 1*S*, 2-*R*; **8a**, 1-*R*, 2-*R*; **1a**, 1-*R*, 2-*R*; **2a**, 1-*S*, 2-*R*. Preliminary calculations showed that for **5a** and **8a** with the axially oriented dimethylamino group the energy profile for rotation about φ_1 (for definition of torsion angles see Fig. 1) is independent of values chosen for φ_2. The two variables were therefore considered separately and full energy profile for both φ_1 and φ_2 was obtained by using the MMP2 driver option with an angle increment of 10°; the φ_2 profile was calculated by setting φ_1 to the biorelevant value of ca. 180° (see below) and then allowed to relax during the minimization procedure. For **6a** and **7a**, where the dimethylamino group is equatorial, the two conformational variables may be considered to be largely independent only for $\varphi_2 > 0°$.

As to the compounds **1a** and **2a**, there are four torsion angles, τ_1-τ_4 (Fig. 1), which define the spatial relationship between the two pharmacophores; hereafter, any conformer will be referred to as (τ_1, τ_2, τ_3, τ_4). Even though the permissible values for τ_1-τ_4 are considerably restricted by the ring closure and steric interactions, a relatively large degree of flexibility still remains; therefore, no attempt was made to explore the conformational space and the energy of the fit conformation was expressed relative to that of the MMP2-optimized X-ray structure. Full-geometry optimization has revealed that the X-ray conformer of both **3** and **4** was close to a local minimum: the X-ray conformers (–51, –112, 82, 140) and (40, 130, –80, 161) of **3**(**1a**) and **4**(**2a**), respectively, gave, due to minimization, conformers (–52, –122, 78, 138) and (42, 143, –84, 148).

Results and Discussion

The purpose of this work was 2-fold: (1) to evaluate and rationalize the local anesthetic activity of geometrical isomers of semirigid derivatives, and (2) to develop a receptor-interaction model for phenylcarbamate class local anesthetics.

Table I. Molecular lipophilicity and relative local anesthetic activity for surface (SLAA) and infiltration (ILAA) anesthesia of compounds **1**-**4**.

Compound	log P^a	SLAA	ILAA
1	2.85	79	103
2	2.90	58	80
3	3.38	109	149
4	3.37	82	122
Cocaine		1	
Procaine			1

[a] log *P* was determined using a calibration curve (straight line, $r = 0.980$) of log *P* (as measured in octanol/water) vs. R_M and was constructed by using some phenylcarbamate derivatives in the work of Gregáň *et al.* (1993a). Thus, R_M is a measure of molecular lipophilicity (equivalent to log *P*, as shown by the high value of the correlation coefficient) and is derived from the TLC R_F values according to the formula $R_M = \log[(1/R_F)-1]$. The TLC experiments were performed under partitioning conditions as described by Gregáň *et al.* (1993b).

Results of the pharmacological evaluation of compounds **1**-**4** or the surface and infiltration anesthesia are listed in Table I. Consistent with the previous finding (Gregáň *et al.*, 1993b) that log P of about **4** is optimal for transport of the drug through the membrane as a major determinant of the *in vivo* activity, both types of anesthesia have consistently indicated higher level of activity for the 2′-butoxy derivatives **3** and **4** relative to the corresponding 2′-propoxy analogues (**1** and **2**). On the other hand, based on the assumption that a precise spatial arrangement of the two pharmacophores is a requirement for activity, we surprisingly found only a small difference in potency between the *cis* and *trans* isomers, with the *cis* isomers being not even twice as potent as the corresponding *trans* ones.

To proceed further, we were prompted to derive a receptor site model of general validity at least for this chemical class of local anesthetics. To achieve

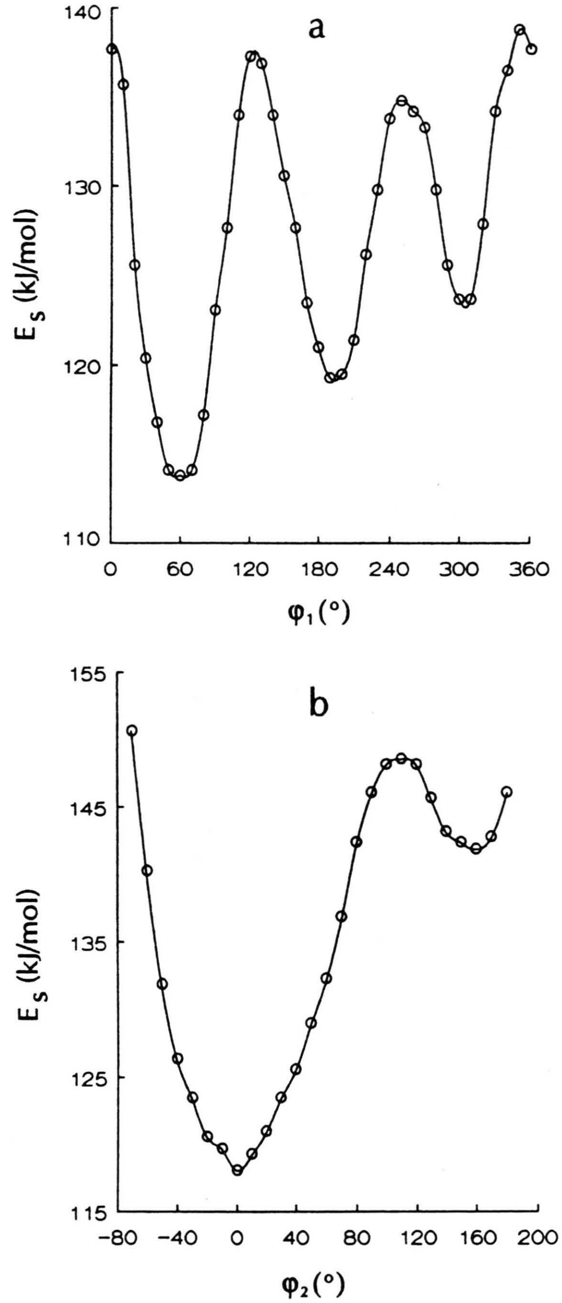

Fig. 2. Steric energy (E_s) profile for rotation about φ_1 (a) and φ_2 (b) for compound **5a** as calculated by the molecular mechanics program MMP2(85) (Allinger, 1985). Torsion angles (φ_1 and φ_2) are defined in Fig. 1.

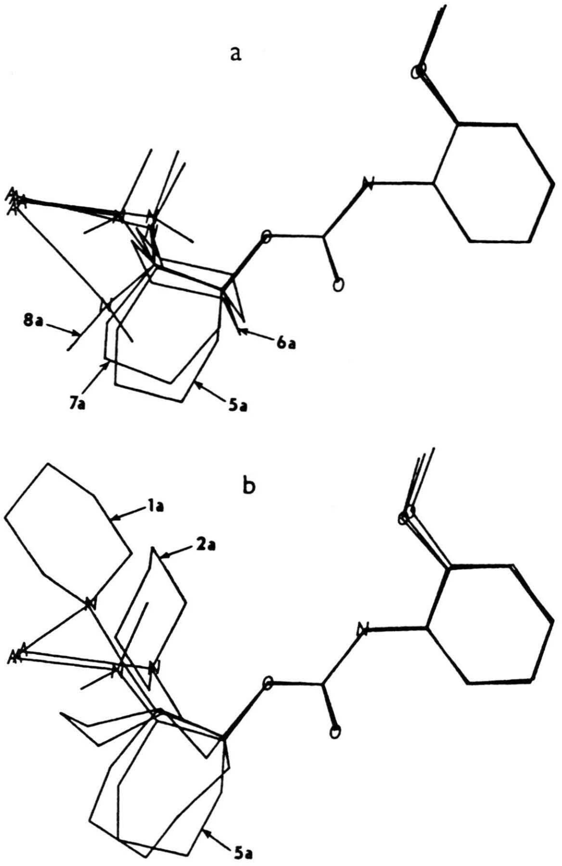

Fig. 3. Stereoview of the superimposition of the four diastereomers **5a**-**8a** (a) and **5a**, **1a**, and **2a** (b) as obtained from the MOLGEN multifitting procedure (see Table II).

this, a set of isomers **5**-**8** with known pharmacological data (Fig. 1) (Beneš *et al.*, 1982) was selected. Initially, it was hypothesized that the phenylcarbamate portion (which is known to be indispensable to activity) of all derivatives considered binds to the receptor in the same orientation and hence can be overlaid in the superimposition studies. As a test of this assumption, we were interested to know whether a correlation exists between activity and the ability of the molecules to hydrogen bond via their protonated amine to an anionic receptor site, mimicked by the dummy atom A_{opt} of **5a**. Thus, the latter compound, being active at the micromolar level, was used as a template in the modeling studies. Its conformational properties with respect to rotations about the two rotatable bonds (φ_1 and φ_2) are shown in Fig. 2. The $\varphi_1 = 60°$ conformations direct the lone pair (N^+–H bond) of the amine nitrogen toward the molecular bulk so that the approach of the N^+–H bond to the H-bond acceptor would be sterically hindered in these conformations. Thus, of the three staggered rotamers of the dimethylamino group, that with $\varphi_1 = 195°$, being 5.0 kJ/mol higher in energy than the global minimum, is the only rotamer with a favourable orientation of its N^+–H bond and was therefore considered to be the receptor-bound conformation; consequently, the conformer ($\varphi_1 = 195°$, $\varphi_2 = 0°$) of **5a** was used as the reference structure in all superimposition studies, the results of which are shown in Fig. 3.

Examination of Fig. 1 and Table II reveals that for **5a**-**8a** there is a satisfactory correlation between the IC_{50} values and the cost in steric energy (ΔE_s) to attain the fit conformation. Nevertheless, the 7.5 kJ/mol of steric energy needed for **8a** to adopt the fit conformation does not adequately account for the 95-fold difference in potency, corresponding to ~ 11.7 kJ/mol difference in free energy of binding ($\Delta G° = RT \ln K_d$, i.e. $5.95 \log K_d$ at 300 K), between **5a** and **8a**. The discrepancy would be removed if one assumes that there is an additional lipophilic binding site (near the amino nitrogen) capable of interacting with the N–R substituent. If this receptor site is located in the upper left part of Fig. 3, then both methyl groups of **5a** fit into this region of space, one methyl for both **6a** and **7a**, while in the case of **8a** neither of the two methyl groups is favourably positioned to come into interaction with the lipophilic site. Considering that each carbon, when dissolving in hydrophobic areas on proteins, contributes on average by ~ 1.7 kJ/mol to the binding energy (Kettmann *et al.*, 1991), then almost a quantitative agreement is obtained between activity and the combined energy term (ΔE_s + ΔE_{lip}) (Table II).

Table II. Relevant geometric and energy results of the superposition studies (shown in Fig. 3).

Comp.	rms[a] [Å]	ΔA_i[b] [Å]	ΔE_s[c]	(ΔE_s+ΔE_{lip})[d]
1a	0.18	0.16	1.7	
2a	0.31	0.28	−4.6	
5a	—	—	0	0
6a	0.21	0.17	3.8	5.5
7a	0.19	0.20	4.6	6.3
8a	0.25	0.30	7.5	10.9

[a] Root mean square (rms) deviation of six atoms of the phenylcarbamate portion which were used as fitting points in the superimposition studies.

[b] For each compound studied a dummy atom (point) A was constructed on the amine nitrogen (see text) and A of the most active derivative **5a** was assumed to mimic the putative hydrogen bond acceptor on the receptor. Then $\Delta A_i = A_{\mathbf{5a}} - A_i$ was calculated and minimized (by rotation on flexible bonds) during the superimposition procedure to assess the ability of the i-th molecule to bring its point A into coincidence with $A_{\mathbf{5a}}$.

[c] Relative steric energy (kJ/mol) of the fit conformation (see text); for compounds **1a** and **2a** relative to the MMP2-calculated X-ray minimum; for compounds **5a**-**8a** relative to the local minimum of the receptor-bound conformation of **5a** which is 5.0 kJ/mol above the global energy minimum.

[d] ΔE_{lip} for compounds **5a**-**8a** stands for a loss of energy (relative to **5a**) of the lipophilic interaction of the two N-methyl substituents and amounts to 1.7 kJ/mol for **6a** and **7a** and to 3.4 kJ/mol for **8a** (see text).

It should be emphasized at this point that the above correlation was obtained by using the phenylcarbamate pharmacophore as an anchor in the molecular modeling studies. This implies that the drug-receptor interaction forces the pharmacophore into a fixed position and orientation which in turn indicates that its binding must occur through at least three noncoplanar points. Knowing that the 2′-alkoxy group is not required at the receptor (Gregáň *et al.*, 1993b), it is very likely that the phenyl ring is involved in (either lipophilic or π-stacking) interaction with an orientationally constrained aromatic residue or perhaps sandwiched between two planar residues; either of these interactions, which are well-known as key contributors to molecular recognition events (Hunter and Saunders, 1990; Pirkle and Pochapsky, 1987; Nogrady, 1988), is expected to be highly dependent on proper position and orientation of the aromatic ring. This, along with the putative hydrogen bond interaction of the carbonyl oxygen with a receptor H-bond donor site, which is known to be highly directional (Taylor *et al.*, 1983) is sufficient to fix the phenylcarbamate moiety in space when the drug is associated with the receptor. In contrast to the sp^2 lone pairs of the carbonyl oxygen, the sp^3 lone pairs in $O{=}\overset{|}{C}{-}O^{-}\cdots H{-}X$ bonds does not show any directionality (Taylor *et al.*, 1983) and this is also shown in Fig. 3, where a range of orientations of the $N^{+}{-}H$ bond is tolerated.

In the next phase of the study, we attempted to relate the anesthetic activity of compounds **1**-**4** (Table I) to their three-dimensional structure using the above binding model. The two isomers **1a** and **2a** were initially superposed on the model in their MMP2-optimized X-ray conformations. As reported earlier (Kettmann *et al.*, 1993a; Kettmann *et al.*, 1993b), the cycloheptane ring of **1a** was found in the C2-half chair conformation, while in **2a** the same ring, due to pseudorotation, assumes the C3-half chair form; as revealed in the initial superimpositions, this conformational flexibility makes it feasible for the two isomers to adopt a similar position of their dummy atom A relative to the phenylcarbamate anchor. Nevertheless, the ΔA values of 0.77 and 1.95 Å for the pairs **5a**(template)/**1a** and **5a**/**2a**, respectively, were still too large to explain the high activity and near equipotency of **1**-**4**. However, molecular modeling has shown that for the *cis* isomer **1a** the fit conformation (–52, –140, 69, 130) (shown in Fig. 3b) is accessible from the MMP2-calculated X-ray minimum (–52, –122, 78, 138) at negligible energy cost (1.7 kJ/mol). More drastic conformational rearrangement is needed for **2a** to attain the fit conformation; however, when the constrained conformation resulting from the fit procedure was allowed to relax by rerunning MMP2 (i.e., without any fitting constraints) it was found that the fit conformation (42, 148, –83, 108) (Fig. 3b) is practically identical with a local minimum lying 4.6 kJ/mol below the MMP2-minimized X-ray conformer (42,143,-84,148). As shown in Fig. 3b, the *cis* isomer **1a** extends its N–R substituent (piperidine ring) beyond that found for other derivatives, suggesting that the pocket that binds R has some degree of flexibility to expand to accommodate one-dimensional variations in the drug.

Although the model presented here was derived on the basis of isomers **5**-**8**, it is compatible not only with compounds **1**-**4** but also with all known, essentially flexible, active drugs of the phenylcarbamate class local anesthetics (results not shown). In this regard, it is interesting to note that attempts to explain the activity of other chemical classes of local anesthetics by this receptor model (or by its enantiomeric image) have consistently failed; this supports the idea (Glówka *et al.*, 1991) that the Na^{+} channel protein can exist in a multitude of discrete conformational states so that different compounds are recognized, either in a one-step (simultaneous) or stepwise ("zipperlike") process, by different receptor conformations. Obviously, the phenylcarbamate class local anesthetics map just one particular state of the protein and the results of this study indicate that in this state, despite the channel itself has large amount of conformational flexibility, the relative position of the two sites that bind the two pharmacophores is quite rigid.

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