

Dedicated to Prof. Dorin N. Poenaru's  
70th Anniversary

## COMPUTER SIMULATIONS OF LOCAL ANESTHETICS MECHANISM: QUANTUM CHEMICAL INVESTIGATION OF PROCAINE

A.-N. BONDAR<sup>1,2,#,\*</sup>, S. SUHAI<sup>2</sup>, J.C. SMITH<sup>1</sup>, P.T. FRANGOPOL<sup>3,\*</sup>

<sup>1</sup>Computational Molecular Biophysics, IWR, University of Heidelberg, Im Neuenheimer  
Feld 368, D-69120 Heidelberg, Germany

<sup>2</sup>Molecular Biophysics Department, German Cancer Research Center, Im Neuenheimer  
Feld 580, D-69120 Heidelberg, Germany

<sup>3</sup>National University Research Council, Schitu Magureanu 1, Bucharest 050025, Romania

# Present address: University of California at Irvine, Department of Physiology and  
Biophysics, School of Medicine, Med. Sci. I, D374, Irvine, CA 92697-4560

\* Corresponding authors: E-mail: nicoleta.bondar@uci.edu (Ana-Nicoleta Bondar);  
pfrangopol@clicknet.ro (Petre T. Frangopol)

(Received February 22, 2007)

*Abstract.* A description at the atomic level of detail of the interaction between local anesthetics, lipid membranes and membrane proteins, is essential for understanding the mechanism of local anesthesia. The importance of performing computer simulations to decipher the mechanism of local anesthesia is discussed here in the context of the current status of understanding of the local anesthetics action. As a first step towards accurate simulations of the interaction between local anesthetics, proteins, lipid and water molecules, here we use quantum mechanical methods to assess the charge distribution and structural properties of procaine in the presence and in the absence of water molecules. The calculations indicate that, in the absence of hydrogen-bonding water molecules, protonated procaine strongly prefers a compact structure enabled by intramolecular hydrogen bonding. In the presence of water molecules the torsional energy profile of procaine is modified, and hydrogen-bonding to water molecules is favoured relative to intra-molecular hydrogen bonding.

*Key words:* mechanism of local anesthetics action; procaine; density functional theory; computer simulations.

## 1. INTRODUCTION

Local anesthetics are chemical substances that cause a local and reversible loss of pain sensation. These chemical compounds diffuse through the lipid membrane and interfere with the functioning of voltage-gated sodium channels, inhibiting the propagation of the action potential (for reviews see, e.g., Refs. [1, 2]). In addition to causing anesthesia, local anesthetics impact other proteins with essential cellular roles, such as, e.g., G $\alpha$ q proteins [3], potassium channel Kv1.1 [4], Ca<sup>2+</sup> ATP-ase [5], kinesin [6], or spectrin [7]. This capability to interfere with the functioning of key proteins renders local anesthetics very attractive for pharmacological applications and, as a consequence, aspects of the local anesthetics action have been studied intensively. Although experiments provided valuable information on the physical chemical properties of local anesthetics and on the interaction between local anesthetics, proteins, and lipid membranes, the mechanism of local anesthesia remains controversial. The recent advancements in computer simulation techniques held promise that questions regarding the physical chemical principles of local anesthetics action can be addressed at the atomic level of detail. But reliable molecular dynamics simulations require an accurate description of the structural properties of the local anesthetic, and of the interactions between the local anesthetic and its environment (e.g., water, lipid molecules, or protein groups). Here, we employ quantum chemical calculations to analyze the charge distribution, the structural and torsional properties of the local anesthetic procaine in the presence and in the absence of hydrogen-bonding water molecules.

Tertiary amine local anesthetics, such as procaine (Fig. 1), have an amphiphilic nature and can exist in protonated and unprotonated forms. The hydrophilic tertiary amine group is usually linked, via an amide or ester linkage, to a hydrophobic aromatic group. Both the tertiary amine and the hydrophobic aromatic group are essential for the local anesthetics action, as the formation of complexes between local anesthetics and their receptors in the lipid membrane involves hydrogen bonding, hydrophobic, and  $\pi$ -electron interactions [8, 9, 10, 11, 12].

An essential question regarding the mechanism of local anesthetics is whether local anesthetics engage in non-specific interactions with the lipid bilayer or, local anesthetics bind to specific sites of the sodium channel. Both these hypothesis have been investigated using various experimental approaches, and it appears that an understanding of the mechanism of local anesthesia requires detailed knowledge of the complex interactions between local anesthetics, lipid, water molecules, and the membrane-embedded sodium channel.

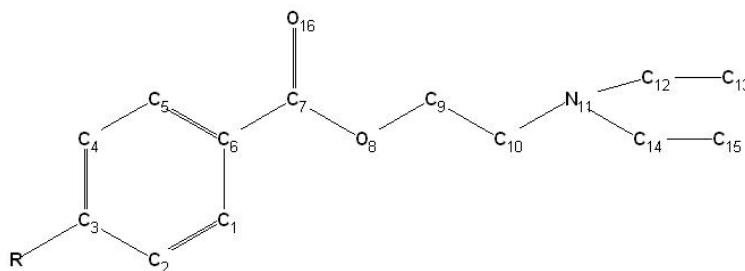


Fig. 1 – Schematic representation of procaine in the unprotonated form. For simplicity, hydrogen atoms are not depicted. R denotes  $-\text{NH}_2$ .

Local anesthetics influence the structural, mechanical, thermodynamical, and dynamical properties of lipid bilayers (reviewed in Ref. [2]; see, e.g., Refs. [13, 14, 15]). The ability of local anesthetics to partition into the hydrophobic environment of model membranes depends on the intrinsic hydrophobicity of the local anesthetic [16], the transient location of the local anesthetic within the lipid membrane depending on the physical-chemical properties of the local anesthetic [17] and on the lipid phase [18]. In the presence of certain hydrophobic ions, the local anesthetic bupivacaine perturbs the mitochondrial membrane to such an extent that there form proton leakage pathways [19].

The interaction between the local anesthetics and sodium channels depends on the channel isoform and on the conformational state of the channel. The functioning of the voltage-gated sodium channels involves protein conformational changes between the resting, open and inactivated states. The resting (closed) state opens in response to depolarization, and then converts into inactive, non-conducting states. The binding affinity of the local anesthetics appears to be higher for the open and inactive states than for the resting state of the channel [20]. The sites for the binding of local anesthetics to the open and inactivated channel might be different [21], and they may become available upon conformational changes of the sodium channel [22, 23]. The binding site for local anesthetics is likely to be located inside the channel pore [24], being lined by the S6 transmembrane segments of the sodium channel (see, e.g., Refs. [10, 23, 25]). Both hydrophobic groups (phenylalanine, leucine, isoleucine) and groups with hydrogen-bonding capability (e.g., asparagines, tyrosine) may be important for local anesthetic binding (see, e.g., Refs. [10, 26]). Protein groups forming the local anesthetics binding site might also interact with ranolazine, a potential antiarrhythmic drug whose structure resembles that of the local anesthetic lidocaine [27].

In addition to the protein groups from the inner pore, groups that are part of the inactivation gate of the sodium channel may engage in specific interactions with local anesthetics. The inactivation gate, which consists of

the loop connecting domains III and IV of the sodium channel, contains a phenylalanine whose  $\pi$ -stacked interactions with the local anesthetic molecule could stabilize the inactivated state [28]. It had also been proposed that local anesthetics may bind to an additional, external site, leading to drug-bound states which retain sodium ion conductivity, albeit with altered channel kinetics [29].

Understanding the interaction between local anesthetics and sodium channels is further complicated by the fact that isoforms of the sodium channels exhibit intrinsic differences in their sensitivity to local anesthetics, and their reaction cycles may also involve various periods of time in which the inactive state of the channel, with which local anesthetics preferentially interact, is available. For example, the cardiac isoform of the sodium channel has a higher binding affinity for lidocaine than, e.g., the skeletal muscle isoform, and is also available in the inactive state for a longer period of time [30]. Specific differences between the amino-acid sequences of two of the S6 segments of the  $\text{Na}_v1.7$  and  $\text{Na}_v1.8$  sodium channels isoforms were suggested to account for the differences in the sensitivity to lidocaine of those two sodium channels [31].

Computer simulations of local anesthetics action are still in an incipient phase. Possible reasons why the interaction between local anesthetics and sodium channels has been little studied by computational approaches reside in the lack of optimized molecular mechanics parameters for the local anesthetic molecules, and of accurate structural information about the sodium channel. Recently, computer modelling of the sodium channel provided useful information on putative conformations of the channel pore, and led to the suggestion that the block of sodium channel might be mainly electrostatic [9]. Given the inaccuracies that could be associated with structural models obtained from homology modelling [32], validation of the electrostatic interactions scenario requires experiments and reliable computer simulations.

Quantum chemical studies assessed the local anesthetics physical chemical properties [33, 34, 35], and aspects of the interaction of local anesthetics with water molecules [33], ions, peptide, and phosphate group models [35]. AM1 quantum mechanical studies in which continuum models were used to describe the solvent found low-energy conformers of the protonated tertiary amine anesthetics characterized by intramolecular hydrogen bonding [34]. This is important, because the molecular size of a local anesthetic may influence the access of the drug to the sodium channel [36] and, moreover, protonated local anesthetics with compact geometries as result of intramolecular hydrogen bonding should be more hydrophobic and hence could enter the lipid membrane [34]. The formation of compact conformers via intramolecular hydrogen bonding could also explain the suggestion that cationic local

anesthetics dissociating from the closed sodium channel might escape into the lipid membrane [37].

The capability of local anesthetics to form inter-molecular hydrogen bonds is another essential property that might determine the structure and dynamics of complexes formed by local anesthetics with water, lipid and protein molecules. Molecular dynamics simulations of lidocaine in a dimyristoyl phosphatidylcholine (DMPC) bilayers indicated strong hydrogen-bonding interactions between a solvent water molecule and the tertiary amine nitrogen of protonated lidocaine [38]. Unlike the neutral form, protonated lidocaine also engages in hydrogen-bonding interactions with the lipid headgroups [38].

Here, the impact of hydrogen-bonding water molecules on the charge distribution and torsional properties of procaine is investigated using Density Functional Theory methods. The results discussed here will be valuable for deriving a set of optimized molecular mechanics parameters to be used in molecular dynamics simulations.

## 2. METHODS

Two different sets of calculations were performed as follows. In the first set of calculations, the charge distribution of the protonated and unprotonated forms of procaine was computed in the absence of water molecules and in the presence of three, and respectively two water molecules. In the case of protonated procain, the three water molecules hydrogen bond to the amide, carbonyl, and tertiary amine groups, whereas in the case of neutral procaine the two water molecules hydrogen bond to the amide and carbonyl groups, respectively. Using the Gaussian03 software [39], the protonated and unprotonated procaine molecules (with and without water molecules) were geometry-optimized at the B3LYP/6-31G\*\* level. The electrostatic potential fitting partial charges were computed using the Chelpg module of Gaussian03.

In the second set of calculations, a search for possible geometries of the protonated procaine molecule was performed using constrained geometry optimizations and then the Conjugate Peak Refinement algorithm (CPR, [40]). The calculations were performed in the absence and in the presence of water molecules, using the approximate Density Functional Theory method SCC-DFTB (self-consistent charge density functional tight binding [41]).

## 3. RESULTS AND DISCUSSION

The action of local anesthetics involves complex interactions between local anesthetics, water, lipid, and protein molecules. In what follows, we

discuss the impact of hydrogen-bonding water molecules on the charge distribution and the preferred geometry of the local anesthetics molecule procaine based on quantum chemical calculations.

The analysis of the partial charge distribution indicates a significant impact of the hydrogen-bonding water molecule on the charge of the tertiary amine nitrogen atom, protonated or unprotonated ( $N_{11}$ ; see Fig. 1 for numbering of the procaine atoms). At the B3LYP/6-31G\*\* level, the partial charge of the neutral amine nitrogen  $N_{11}$  is  $-66.837 \times 10^{-2}e$  in the presence of hydrogen-bonding water, as compared to  $15.429 \times 10^{-2}e$  in the absence of water. In the case of protonated procaine, the partial charge on the  $N_{11}$  atom is  $-13.777 \times 10^{-2}e$  in the presence of water, and  $25.832 \times 10^{-2}e$  in the absence of the hydrogen-bonding water molecule. This effect of water molecules on the charge distribution of procaine will need to be accounted for in molecular dynamics simulations of procaine.

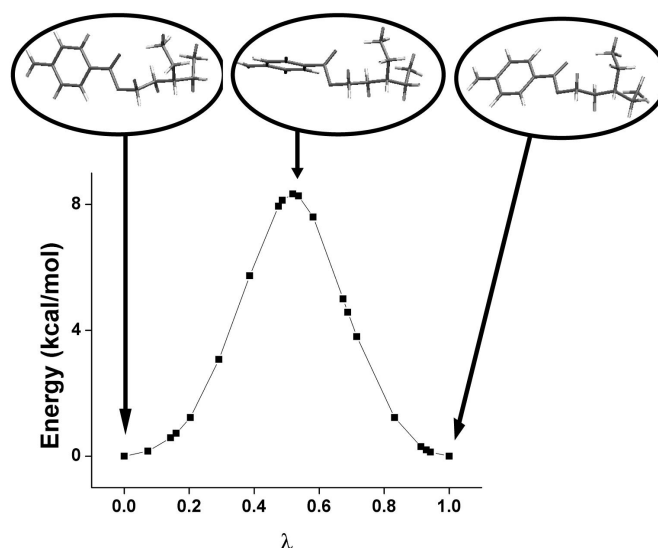


Fig. 2 – Minimum-energy profile for torsion around the  $C_6-C_7$  bond in the absence of hydrogen-bonding water molecules. The reaction coordinate  $\lambda$ , gives the sum of the change in all atomic coordinates along the path, measured as root-mean-squared differences [40].  $\lambda$  was normalized to the total length of the path. The  $C_5-C_6-C_7-O_8$  dihedral angle is  $-197.7^\circ$  at  $\lambda = 0$ , and  $0.8^\circ$  at  $\lambda = 1$ . The energies are SCC-DFTB-optimized values (in kcal/mol), taken relative to the energy value at  $\lambda = 0$ . Insets show the geometry of the procaine molecule along the pathway.

The energy profile for twisting around the  $C_6-C_7$  bond indicates that the intrinsic energy barrier could allow moderate bond twisting without a

significant energy cost. For example, in the gas-phase, it costs  $\sim 3$  kcal/mol to twist the C<sub>6</sub>–C<sub>7</sub> bond by 33 degrees ( $\lambda = 0.3$  in Fig. 2), and  $\sim 6$  kcal/mol to twist the C<sub>6</sub>–C<sub>7</sub> bond by 53 degrees ( $\lambda = 0.4$  in Fig. 2).

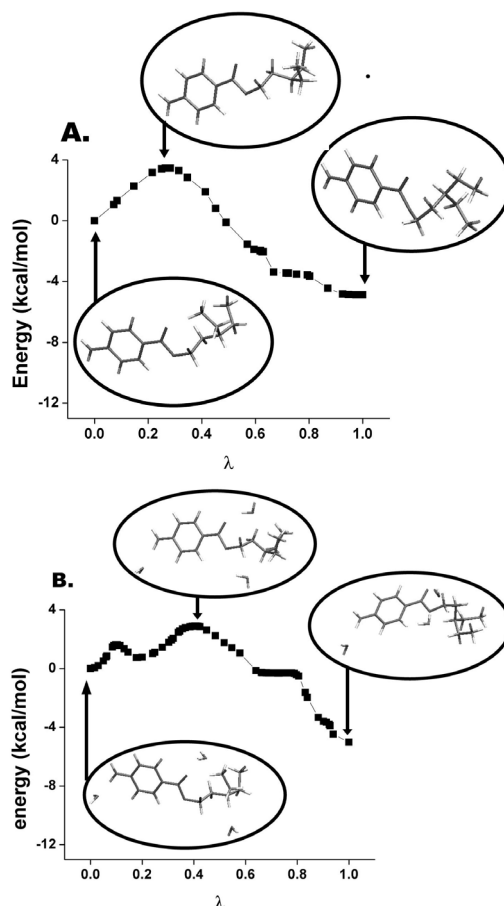


Fig. 3 – Minimum-energy profile for torsion around the C<sub>9</sub>–C<sub>10</sub> bond in the absence (A) and in the presence (B) of hydrogen-bonding water molecules. In panel (A), the O<sub>8</sub>–C<sub>9</sub>–C<sub>10</sub>–N<sub>11</sub> dihedral angle is 177.2° at  $\lambda = 0$ , and  $-26.7^\circ$  at  $\lambda = 1$ . In panel (B), the values of the dihedral angle are 178.7° at  $\lambda = 0$  and  $-41.4^\circ$  at  $\lambda = 1$ .

In the absence of water molecules, intra-molecular hydrogen bonding between the carbonyl and the protonated amine nitrogen group leads to the formation of a more compact geometry that is  $\sim 5$  kcal/mol lower in energy than the extended geometry. In the lowest-energy conformer ( $\lambda = 1$  in Fig. 3A), the distance between the protonated tertiary amine nitrogen (N<sub>11</sub>)

and the carbonyl oxygen ( $O_{16}$ ) is 2.7 Å, and the  $N_{11}-O_{16}-H_{11}$  angle is 14.8°. Breaking this strong intra-molecular hydrogen via rotation around the  $C_9-C_{10}$  bond requires 8.3 kcal/mol (Fig. 3A). Such a geometry characterized by intra-molecular hydrogen bonding could allow incorporation of protonated procaine in the hydrophobic environment of a lipid membrane, or in a hydrophobic protein pocket.

Relative to the gas-phase calculation above, the presence of hydrogen-bonding water molecules changes significantly the energy profile and structural changes associated with the rotation around the  $C_9-C_{10}$  bond of protonated procaine. Relocation of water molecules accompanies the rotation around the  $C_9-C_{10}$  bond, such that the hydrogen bond with the carbonyl and tertiary amine nitrogen groups are preserved. As a consequence, there is no longer a direct, intra-molecular hydrogen bond between these two groups. In the lowest-energy conformer ( $\lambda = 1$  in Fig. 3B), the distance between the  $O_{16}$  and  $N_{11}$  atoms is 3.6 Å, which is too long for a hydrogen bond. Instead, the carbonyl and tertiary amine nitrogen groups engage in strong (2.7 – 2.8 Å) hydrogen bonds with water molecules (see insets in Fig. 3B).

#### 4. CONCLUSION

The procaine local anesthetic is a relatively flexible molecule whose geometry can be significantly influenced by water molecules. In the absence of water, the lowest-energy geometry of protonated procaine is characterized by a strong intra-molecular hydrogen bond. This hydrogen bond is no longer formed in the presence of water molecules. In that latter case, the tertiary amine nitrogen and the carbonyl groups hydrogen bond to water molecules. The results discussed here will be useful for deriving optimized molecular mechanics parameters for molecular dynamics simulations.

#### REFERENCES

1. Catterall, W.A., *Common modes of drug action on  $Na^+$  channels: local anesthetics, antiarrhythmics and anticonvulsants*, TIPS, **8**, 57–65 (1987).
2. Frangopol, P.T., and Mihailescu, D., *Interactions of some local anesthetics and alcohols with membranes*, Colloids and Surfaces B: Biointerfaces, **22**, 3–22 (2002).
3. Hollmann, M.W., DiFazio, C.A., and Durieux, M.E., *Ca-signaling G-protein-coupled receptors: a new site of local anesthetic action?*, Reg. Anesthesia and Pain Med., **26**, 565–571 (2001).
4. Elliot, A.A., Harrold, J.A., Newman, J.P., and Elliot, J.R., *Open channel block and open channel destabilization: contrasting effects of phenol,  $TEA^+$  and local anesthetics on  $Kv1.1$   $K^+$  channels*, Toxicol. Lett., **100–101**, 277–285 (1998).

5. Wolosker, H., Pacheco, A.G.F., and de Meis, L., *Local anesthetics induce fast Ca<sup>2+</sup> efflux through a nonenergized state of the sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase*, J. Biol. Chem., **267**, 25, 5785–5789 (1992).
6. Miyamoto, Y., Muto, E., Mashimo, T., Iwane, A.H., Yoshiya, I., and Yanagida, T., *Direct inhibition of microtubule-based kinesin motility by local anesthetics*, Biophys. J., **78**, 940–949 (2000).
7. Mondal, M., and Chakrabarti, A., *The tertiary amine local anesthetic dibucaine binds to the membrane skeletal protein spectrin*, FEBS Lett., **532**, 396–400 (2002).
8. Fraceto, L.F., Oyama, S. Jr., Nakaie, C.R., Spisni, A., de Paula, E., and Pertinhez, T.A., *Interaction of local anesthetics with a peptide encompassing the IV/S4-S5 linker of the Na<sup>+</sup> channel*, Biophys. Chem., **123**, 29–39 (2006).
9. Lipkind, G.M., Fozzard, H.A., *Molecular modelling of local anesthetic drug binding by voltage-gated sodium channels*, Mol. Pharmacol., **68**, 1611–1622 (2005).
10. Ragsdale, D.S., McPhee, J.C., Scheuer, T., and Catterall, W.A., *Molecular determinants of state-dependent block of Na<sup>+</sup> channels by local anesthetics*, Science, **265**, 1724–1728 (1994).
11. Sax, M., and Pletcher, J., *Local anesthetics: significance of hydrogen bonding in mechanism of action*, Science, **166**, 1546–1548 (1969).
12. Zamponi, G.W., and French, R.J., *Amine blockers of the cytoplasmic mouth of sodium channels: a small structural change can abolish voltage dependence*, Biophys. J., **67**, 1015–1027 (1994).
13. Baciú, M., Holmes, M.C., and Leaver, M.S., *Morphological transitions in model membrane systems by the addition of anesthetics*, J. Phys. Chem., B **111**, 909–917 (2007).
14. Bötner, M., and Winter, R., *Influence of the local anesthetic tetracaine on the phase behaviour and the thermodynamic properties of phospholipid bilayers*, Biophys. J., **65**, 2041–2046 (1993).
15. Yun, I., Cho, E.-S., Jang, H.-O., Kim, U.-K., Choi, C.-H., Chung, I.-K., Kim, I.-S., and Wood, W.G., *Amphiphilic effects of local anesthetics on rotational mobility in neuronal and model membranes*, Biochim. Biophys. Acta, **1564**, 123–132 (2002).
16. Matsuki, H., Kaneshina, S., Kamaya, H., and Ueda, I., *Partitioning of charged local anesthetics into model membranes formed by cationic surfactant: Effect of hydrophobicity of local anesthetic molecules*, J. Phys. Chem., B **102**, 3295–3304 (1998).
17. Fraceto, L.F., Spisni, A., Schreier, S., and de Paula, E., *Differential effects of uncharged aminoamide local anesthetics on phospholipid bilayers, as monitored by 1H-NMR measurements*, Biophys. Chem., **115**, 11–18 (2005).
18. Hutterer, R., Kräamer, K., Schneider, F.W., and Hof, M., *The localization of the local anesthetic tetracaine in phospholipid vesicles: a fluorescence quenching and resonance energy transfer study*, Chem. Phys. Lipids, **90**, 11–23 (1997).
19. Terada, H., Shima, O., Yoshida, K., and Shinohara, Y., *Effects of the local anesthetic bupivacaine on oxidative phosphorylation in mitochondria*, J. Biol. Chem., **265**, 7837–7842 (1990).
20. Ragsdale, D.S., Scheuer, T., and Catterall, W.A., *Frequency and voltage-dependent inhibition of type IIA Na<sup>+</sup> channels, expressed in a mammalian cell line, by local anesthetic, antiarrhythmic, and anticonvulsant drugs*, Mol. Pharmacol., **40**, 756–765 (1991).
21. Zamponi, G.W., and French, R.J., *Dissecting lidocaine action: diethylamide and phenol mimic separate modes of lidocaine block of sodium channels from heart and skeletal muscle*, Biophys. J., **65**, 2335–2347 (1993).

22. Smith, J.A.M., Amagasu, S.M., Hembrador, J., Axt, S., Chang, R., Church, T., Gee, C., Jacobsen, J.R., Jenkins, T., Kaufman, E., Mai, N., and Vickery, R.G., *Evidence for a multivalent interaction of symmetrical, n-linked, lidocaine dimers with voltage-gated Na<sup>+</sup> channels*, *Molecular Pharmacology*, **69**, 921–931 (2006).
23. Yarov-Yarovoy, Brown, J., Sharp, E.M., Clare, J.J., Scheuer, T. and Catterall, W.A., *Molecular determinants of voltage-dependent gating and binding of pore-blocking drugs in transmembrane segment IIS6 of the Na<sup>+</sup> channel  $\alpha$  subunit*, *J. Biol. Chem.*, **276**, 20–27 (2001).
24. Cahalan, M.D., and Almers, W., *Interactions between quaternary lidocaine, the sodium channel gates, and tetrodotoxin*, *Biophys. J.*, **27**, 39–56 (1979).
25. Yarov-Yarovoy, McPhee, J.C., Idsvoog, D., Pate, C., Scheuer, T. and Catterall, W.A., *Role of amino acid residues in transmembrane segments IS6 and IIS6 of the Na<sup>+</sup> channel  $\alpha$  subunit in voltage-dependent gating and drug block*, *J. Biol. Chem.*, **277**, 35393–35401 (2002).
26. Li, H.-L., Galue, A., Meadows, L., and Ragsdale, D.S., *A molecular basis for the different local anesthetic affinities of resting versus open and inactivated states of the sodium channel*, *Mol. Pharmacol.*, **55**, 134–141 (1999).
27. Fredj, S., Sampson, K.J., Liu, H., and Kass, R.S., *Molecular basis of ranolazine block of LQT-3 mutant sodium channels: evidence for site of action*, *British J. Pharmacol.*, **148**, 16–24 (2006).
28. Kuroda, Y., Ogawa, M., Nasu, H., Terashima, M., Kasahara, M., Kiyama, Y., Wakita, M., Fujiwara, Y., and Nakagawa, T., *Locations of local anesthetic dibucaine in model membranes and the interaction between dibucaine and a Na<sup>+</sup> channel inactivation gate peptide as studied by <sup>2</sup>H- and <sup>1</sup>H-NMR spectroscopies*, *Biophys. J.*, **71**, 1191–1207 (1996).
29. Baumgarten, C.M., Makielski, J.C., and Fozzard, H.A., *External site for local anesthetic block of cardiac Na<sup>+</sup> channels*, *J. Mol. Cell. Cardiol.*, **23**, 85–93 (1991).
30. Wang, D.W., Nie, L., George, A.L., and Bennett, P.B., *Distinct local anesthetic affinities in Na<sup>+</sup> channel subpores*, *Biophys. J.*, **70**, 1700–1708 (1996).
31. Chevrier, P., Vijayaragavan, K., and Chahine, M., *Differential modulation of Na<sub>v</sub>1.7 and Na<sub>v</sub>1.8 peripheral nerve sodium channels by the local anesthetic lidocaine*, *British J. Pharmacol.*, **142**, 576–584 (2004).
32. Oliveira, L., Hulsén, T., Hulsik, D.L., Paiva, A.C.M., and Vriend, G., *Heavier-than-air flying machines are impossible*, *FEBS Lett.*, **564**, 269–273 (2004).
33. Bernardi, R.C., Gomes, D.E.B., Pascutti, P.G., Ito, A.S., and Ota, A.T., *Theoretical studies on water-tetracaine interaction*, *Int. J. Quantum Chem.*, **106**, 1277–1282 (2006).
34. Delphino, J., Neto, D.M., and De Alencastro, R.B., *Theoretical studies on local anesthetics: procaine, lidocaine, tetracaine, bupivacaine, and dibucaine – neutral and mono-protonated*, *Int. J. Quantum Chem.*, **61**, 959–980 (1997).
35. Remko, M., and Scheiner, S., *Ab initio investigation of interactions between models of local anesthetics and receptor: complexes involving amine, phosphate, amide, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Cl<sup>-</sup>*, *J. Pharm. Sci.*, **77**, 304–308 (1988).
36. Courtney, K.R., *Why do some drugs preferentially block open sodium channels?*, *J. Mol. Cell. Cardiol.*, **20**, 461–464 (1988).
37. Chernoff, D.M., and Strichartz, G.R., *Kinetics of local anesthetic inhibition in neuronal sodium currents. pH and hydrophobicity dependence*, *Biophys. J.*, **58**, 69–81 (1990).

- 
38. Högberg, C.-J., Malianik, A., Lyubartsev, A.P., *Dynamical and structural properties of charged and uncharged lidocaine in a lipid bilayer*, *Biophysical Chemistry*, **125**, 416–424 (2007).
  39. Frisch, M.J. *et al.*, *Gaussian 03*. Gaussian, Inc., Wallingford CT, 2004.
  40. Fischer, S., and Karplus, M., *Conjugate peak refinement: an algorithm for finding reaction paths and accurate transition states in systems with many degrees of freedom*, *Chem. Phys. Lett.*, **194**, 252–261 (1992).
  41. Elstner, M., Porezag, D., Jungnickel, G., Elsner, J., Haugk, M., Frauenheim, T., Suhai, S., and Seifert, G., *Self-consistent-charge density-functional tight-binding method for simulations of complex materials properties*, *Phys. Rev.*, B **58**, 7260–7268 (1998).