Biophys J BioFAST, published on August 24, 2007 as doi:10.1529/biophysj.107.104208

Effect of Local Anesthetic Lidocaine on Electrostatic Properties of a Lipid Bilayer

Carl-Johan Högberg and Alexander P. Lyubartsev

Division of Physical Chemistry, Arrhenius Laboratory, Stockholm University
Stockholm, SE-10691, Sweden

Abstract

The influence of local anesthetic lidocaine on electrostatic properties of a lipid membrane bilayer was studied by molecular dynamics simulations. The electrostatic dipole potential, charge densities, and orientations of the headgroup angle have been examined in presence of different amounts of charged or uncharged forms of lidocaine. Important differences of the membrane properties caused by the presence of the both forms of lidocaine are presented and discussed. Our simulations have shown that both charged and uncharged lidocaine cause almost the same increase of the dipole electrostatic potential in the middle of membrane though for different reasons. The increase, being about 90 mV for 9 mol % of lidocaine and 220 mV for 28 mol% of lidocaine, is of the size which may affect the functioning of voltage-gated ion channels.

Keywords: membranes; DMPC; lidocaine; molecular dynamics; electrostatics

Introduction

Local anesthetics are a well known group of pharmaceutical agents used to relieve pain in specific parts of the organism, inhibiting propagation of signals along the nerves. Despite the fact that local anesthetics have a very important field of application and that they have been used in medical treatment for more than fifty years, the molecular mechanism of their action remains almost unknown (1). A logical extension of the observations by Meyer and Overton (2,3), that the therapeutic potency of anesthetics is correlated with the partition coefficient in olive oil, is that local anesthetics act by targeting the cell membrane. It is known experimentally that anesthetic molecules are able to block Na^+ ion channels in neuronal cells (4-7), thus one can suggest that the anesthetics cause such changes in the membrane or in the membrane proteins which affect functioning of ion channels. During the last decades, protein-oriented theories, claiming that binding of anesthetic molecules to specific binding sites in membrane protein is responsible for the anesthetic effect, have become prevailing (8-11), and a binding site of some local anesthetics in the voltage gated sodium channel has been proposed (12). On the other side, the strong focus from the protein oriented theories on an anesthetic mechanism in terms of local anesthetic interaction with a binding site, not taking the lipid surrounding into account, have been criticized(13,14).

Several different mechanisms of how the presence of local anesthetics in lipid bilayers can modulate conductivity of ion channels have been suggested. One of the plausible mechanisms is related to the electrostatic dipole potential which arises due to oriented dipoles at the membrane-water interface and changes of which upon addition of anesthetics could trigger voltage-gated ion channels (13). Among other discussed mechanisms are changes in the bilayer lateral pressure profile which could shift the equilibrium between the active and inactive forms of membrane proteins (13,15), increase of membrane fluidity (manifested also in the decrease of the phase transition temperature (14)), changes in the lipid hydration (14) and specific hydrogen bond formation (16).

Lidocaine is one of the most common amide-type of local anesthetics. In aqueous solution lidocaine exists usually as a mixture of charged and uncharged species, with a pKa value estimated as 7.9 (17,18). It is believed that the charged form is responsible for the therapeutic action (8). It has been measured that the membrane-water partition coefficient of the uncharged form is by 1.15 higher that that of the charged form (17), which indicates that inside membranes, the balance between the charged and uncharged forms is shifted in favor of the uncharged species. The role of the neutral form of lidocaine may thus also be important due to its higher ability to penetrate inside membranes (18).

In this article we use molecular dynamics simulations to investigate the effect from the charged and uncharged forms of lidocaine on the electrostatic properties of a model lipid membrane. Molecular dynamics simulations enable us to get a very detailed picture of the molecular events and thus provide us with an unique tool to understand phenomena at the molecular level. During the last decade, molecular dynamics have been extensively used to study many properties of lipid bilayers (19-24) including the cases when bilayer associated molecules were present (25-27).

In our previous work (28), we have studied preferential location and orientation of the charged and uncharged lidocaine in DMPC (dimyristoylphosphatidylcholine) bilayer as well as its hydration properties. The analysis of the present work is concentrated on the changes in the lipid bilayer which are caused by the both forms of lidocaine and which are of importance for the membrane electrostatic properties. In addition to the simulations performed in the previous work (28), simulations at three times higher lidocaine concentration have been

carried out and analyzed in order to highlight the effect on membrane caused by the presence of lidocaine.

Computational Methods

Five different lipid bilayer systems, each consisting of 128 DMPC lipids and 3655 water molecules were simulated. In addition to the molecules mentioned above, two of the systems contained also either 12 charged or 12 uncharged lidocaine molecules, and two other systems contained either 36 charged or 36 uncharged lidocaine molecules. Molecular structures of DMPC and the two forms of lidocaine are shown in Figure 1. In order to keep electroneutrality, 12 or 36 Cl^- ions were added to the corresponding systems with charged lidocaine. One system containing a pure fully hydrated DMPC bilayer was simulated as a reference. The molecules were described within the united atom GROMOS force field (except the polar H atom on charged lidocaine which was described explicitly), with the interaction parameters included into GROMACS simulation package (29) . The temperature was set to 313 K and the pressure to 1 bar. The long-range electrostatic forces were treated using the Particle Mesh Ewald (PME) technique (30). Further details on the simulation setup, partial atom charges on lidocaine, system preparation, etc can be found in our previous paper (28).

All simulations containing lidocaine were run 100 ns from the starting conditions. The last 50 ns were used for trajectory analysis. The reference system, with a start configuration taken from a previous well equilibrated simulation (24), was simulated 50 ns from which the last 45 ns were used for trajectory analysis. All simulations were carried out using GROMACS v.3.2 simulation package (29).

For calculation of the electrostatic potential we start from the Poisson equation:

$$\epsilon\epsilon_0\nabla^2\Phi(r)=-\rho(r) \qquad (1)$$

Here $\Phi(r)$ is the electrostatic potential and $\rho(r)$ is the charge density. The dielectric constant ε in atomistic simulations is set to 1.

Due to translational symmetry in X- and Y- direction, the electrostatic potential and the charge density depend only on Z-coordinate. From a simulation we can construct the charge distribution by slicing the Z- direction of the membrane in thin slices and sum for all partial atom charges in each slice. By using the boundary conditions $\Phi(z_0)=0$ and $d\Phi(z)/dz|_{z=z_0}=0$ in reference point z_0, here chosen to be in the middle of the water layer, we can get the electrostatic potential by integrating the Poisson equation over the box z-coordinate:

$$\Phi(z)=-\frac{1}{\epsilon_0}\int_{z_0}^{z}dz'\int_{z_0}^{z'}\rho(z'')dz'' \qquad (2)$$

Results and discussion

Area per lipid

The average area per lipid is a fundamental property of lamellar bilayer systems. Many other properties depend on it in some extent. Here we use the area per lipid to monitor equilibration of the simulated systems. The time evolution of the area per lipid for each of the simulated systems is shown in Figure 2. No visible trends are seen. Block averaging over the last 50ns of the trajectory shows no drift in the evolution of the area per lipid for the simulated systems and consequently the systems were regarded to be in equilibrium.

In Table 1 we present mean values of the calculated areas per lipid. Simulations in the presence of lidocaine show larger areas per lipid and the previously noticed trend that the area per lipid in the system with charged lidocaine is slightly smaller than in the corresponding system with uncharged lidocaine (28) holds even for larger lidocaine concentration.

Electrostatic Potential

The electrostatic potential across membrane is an important property of lipid bilayers which may be relevant for understanding of mechanisms behind the functioning of ion channels. The potential arises due to specific preferential orientations of the lipid headgroup dipoles and water dipoles at the membrane-water interface. By this reason it is often referred to as the bilayer dipole potential. Presence of ions and other charged species affects the electrostatic potential too.

In Figure 3, the total electrostatic potential is presented for all five simulations. In Table 1, the values of the potential in the middle of membrane, and the maximum values of the potential are given. The overall picture is quite well in agreement with previous simulations using GROMACS force field, showing positive dipole potential 500-600 mV in the middle of bilayer (26,31). Accurate experimental determination of the dipole potential is difficult and different sources report different values. Many of them provide values of the potential in the middle of membrane in the range 300-800 mV (32). Other experimental studies have suggested a lower value for the dipole potential in the range 220-280 mV (33), while a value of 510 mV has been recently reported for diphytanoylphosphatidylcholine (ester-DPhPC) membrane (34) which is close to our result for the reference system.

It can be seen that both charged and uncharged lidocaine has a well pronounced influence on the potential. In the headgroup region, one can see a slight decrease of the electrostatic potential by approximately 30 mV for the uncharged lidocaine at the both concentrations. For the charged systems the potential in the headgroup region is increased by 46mV and 76mV for the simulations with 12 and 36 lidocaine molecules respectively. More interesting is the change of the potential for the hydrocarbon region of the lipid tails. The striking result is that the potentials in the middle part of the bilayer are almost the same for charged and uncharged forms of lidocaine at the both concentrations. Comparing to the reference bilayer, the potential is increased by about 93 mV for simulations with 12 lidocaine molecules and by 220 mV for simulations with 36 lidocaine molecules.

The fact that positively charged lidocaine increases the electrostatic potential inside membrane is quite natural. It is remarkable that the uncharged lidocaine does the same. In order to get more insight into this effect, we displayed contributions to the electrostatic potential from DMPC, water, lidocaine and Cl^- ions, see Figure 4. For the reference membrane the resulting potential is obtained as a sum of a positive potential from water and a

negative one from lipids, each of contributions exceeding the resulting potential by about one order of magnitude. In the presence of charged lidocaine, a strong positive contribution appears from the positive lidocaine and negative Cl^- ions, which in the middle of membrane reaches the values of 3.42 V and 7.68 V for the systems with 12 and 36 lidocaine$^+$ respectively (red filled symbols in Fig.4). Simultaneously, one can observe a clear decrease of the potential coming from both DMPC and water (red solid and dashed lines with filled symbols in comparison with the corresponding black lines). For DMPC, there is a decrease by 1.85 V and 4.06 V and for water by 1.45 V and 3.36 V for 12 and 36 lidocaine$^+$ respectively. For water a small dip in the potential can be also seen at the bilayer interface. The dip is more pronounced for the system with 36 lidocaine molecules. It reflects the change in preferential orientation of water dipoles in the outer surface area of the membrane due to their interactions with Cl^- ions. Most of the observed changes of the potential coming from different components cancel each other, resulting in a rather moderate increase of the total electrostatic potential.

In the case of uncharged lidocaine, the picture is different. Neither water nor DMPC contributions to the electrostatic potential change noticeably (compare black lines without symbols and blue lines with open symbols in Fig. 4). To illustrate this more clearly, in Fig.5 we displayed contribution to the dipole potential coming from the uncharged lidocaine only (green lines with diamonds), in comparison with the contribution of water and lipids to the total change of the potential (black lines without symbols). It is clear that almost the whole contribution to the total change of the dipole potential comes from the lidocaine alone. The most strongly charged atom on a neutral lidocaine molecule is the negatively charged carbonyl oxygen (the charge is -0.41), while the rest of the molecule has mainly a weak positive charge. In our previous work (28) we found that the preferential location of the uncharged lidocaine is just below the headgroups with orientation parallel to the bilayer surface. In this coordination, the carbonyl oxygen can orient itself interacting favorably with polar atoms of the headgroups, while the "back" side of lidocaine interact with the upper parts of the apolar lipid tails. Our analysis shows that the most probable value of the angle between carbonyl CO vector of the uncharged lidocaine and bilayer normal lies between 30 and 40 degrees. Such preferential orientation of the uncharged lidocaine may create a small but noticeable contribution into the total electrostatic potential.

Charge density

Since the electrostatic potential is determined by the distribution of different charged groups, in order to get better insight into origin of the observed changes we have in Figure 6 plotted the charge density for different components as a function of the box z-coordinate. For the reference system, the charges from the choline and phosphate groups are compensated by the presence of water. The water contribution to the charge density (dashed lines) is slightly greater than that of the lipids (solid lines), which leads to a total positive potential in the middle of membrane. One can see that the density distribution from the positively charged lidocaine (red filled squares without connection lines) is not overlapping with the negative distribution from Cl^- ions (red filled squares with dot-dashed line). This “double layer” creates a large positive potential if one considers contribution from lidocaine$^+$ and Cl^- only (see Fig.4). However, the positive charge density of the charged lidocaine is largely compensated by the change of the negative charge density from the lipid phosphate groups, while the negative contribution of Cl- ions is mostly compensated by water and choline groups of the lipids. Such compensation of charges can be interpreted as dielectric screening of charged species (lidocaine$^+$ and Cl^-) by dipoles of water and lipid headgroups. From the numerical data cited in the previous section, the increase of the total electrostatic potential is reduced by factor 37 for 12 lidocaine and by factor 35 for 36 lidocaine molecules, comparing with the

increase coming from the charged components only (charged lidocaine and Cl ions). The cited above factor can be viewed as an effective dielectric permittivity of the water-lipid interface, and one can see that the dielectric response is nearly linear in the considered concentration range.

In the case of uncharged lidocaine molecules, the water and lipid charge densities remain mostly unperturbed, and the major part of the change of the total electrostatic potential comes from a fluctuation of the lidocaine charge density only, which is seen in Figure 6 as open blue squares in the range 6 - 14 Å from the bilayer center. It is just this density which creates a positive change of the total dipole potential, while water and lipids do not contribute to the change of the dipole potential in the middle of membrane, as it is seen in Fig.5.

The observed changes of the electrostatic potential are in the same magnitude range as the transmembrane potential for a cell membrane in vivo (32) already in the system with 12 lidocaine molecules (about 9 mol% concentration). This indicates that such changes could be a plausible mechanism for the action of local anesthetics. By changing distribution of the potential inside the membrane, the neuron may be blocked from reaching its threshold value and thus prevented from working properly. Note also that a positive change of the electrostatic potential in the middle of membrane of the order of a few *kT* units (which can also propagate inside an ion channel due to the long-range character of electrostatic interactions) creates an additional energy barrier for cations to pass through membrane.

Headgroup Angle

It is clear from the data presented above that the most significant changes in the bilayer due to addition of lidocaine occur in the headgroup region. Also, behavior of the headgroup dipoles is the main factor behind the membrane electrostatics. We therefore analyzed how the presence of the lidocaine affects the angular distribution of the phosphorus-nitrogen (P-N) vector relative to the bilayer normal, which is assumed to be parallel to the Z-axis of the simulation box. Figure 7 shows the distribution of the P-N vector for each monolayer separately relative to the normal vector directed out of the bilayer. Average values of the P-N tilt angle (the angle between P-N vector and the bilayer normal) are also given in Table 1.

From the figure it can be seen that the uncharged lidocaine has almost no influence on the orientation of the headgroup. The computed average headgroup tilt angle has a value of 79.8 degrees for the reference bilayer, to be compared with 79.0 degrees for the system with 12 and 36 uncharged lidocaines, the difference being of the order of the statistical uncertainty. For the systems with charged lidocaine the effect is more dramatic: the average tilt angle decreases to 72 degrees for 12 lidocaine$^+$ and further down to 60 degrees for 36 lidocaine$^+$. The similar trends are seen in the angular distribution: while the uncharged lidocaine leaves the distributions almost intact, centered at the angle almost parallel to the membrane surface with a slight preference to the direction out of membrane, the charged lidocaine causes noticeable reorientation of the headgroup vectors towards the water phase. Previously, a change of the P-N angle has been suggested as an example of a "molecular voltmeter" (35) defining the membrane dipole potential. If we come back to the dipole potential profiles (Fig. 3), we see that in the headgroup region, up to the ester groups, the dipole potential for systems with the uncharged lidocaine mostly coincides with that of the reference system, while for the charged lidocaine we see an increase of the dipole potential in the headgroup region. This effect clearly correlates with the observed changes in the P - N angle and is in fact not very surprising considering the ionic distribution in water near the bilayer surface, which attracts the positively charged choline groups. Under this circumstances it is energetically advantageous for the headgroup to change its orientation. Moreover, the effect from repulsion

between the positive charge on lidocaine molecules, located at the level of phosphate and ester groups, and the positive charge on the choline group makes the decrease of the angle favorable. The uncharged lidocaine is coordinated mostly under the ester groups and in the upper parts of the lipid tails, and do not affect charge distribution in the headgroup region, causing increase of the electrostatic potential only in the tail region of the membrane. The observed behavior of the headgroup tilt angle is in agreement with the presented above results for the charge distribution and the electrostatic potential.

Conclusions

In this article we have examined the influence of charged and uncharged lidocaine on electrostatic properties of a lipid bilayer which may be of importance for understanding of lidocaine anesthetic action. We have analyzed the electrostatic potential, charge distributions and the headgroup tilt angle. All examined properties showed significant changes of different character in the presence of either charged or uncharged lidocaine molecules.

The dipole electrostatic potential was found to be affected by the presence of the both forms of lidocaine. A very interesting observation is that the electrostatic potential in the lipid tail region turned out to be almost the same for both charged and uncharged lidocaine at equal concentrations. The mechanism of the change of the electrostatic potential is however very different for the two forms. The charged lidocaine, together with neutralizing Cl- ions, has a strong influence on the behavior of the lipid headgroups, leading to decrease of the tilt of the phosphorus - nitrogen dipole vector and generally causing a serious rearrangement of the charges of all molecular species involved. Most of changes in the charge distributions cancel however each other, resulting in a moderate increase of the total electrostatic potential inside the membrane. The uncharged lidocaine keeps the lipid structure and associated charge distribution almost intact. The total electrostatic potential in the middle of membrane is increasing in this case due to partial charges on the lidocaine itself, with the main contribution from dipole moment of the carbonyl group.

Also for the headgroup angle we see significant changes in the presence of charged lidocaine that could influence membrane protein functioning. We note that similar effect, including change of the headgroup tilt angle and increase of the dipole potential, have been observed for cationic lipids (36) which are not known to have anesthetic effects. Thus there can be other molecules that do not cause anestesia but do cause similar molecular effects. On the other hand, there is an observation that addition of positively charged lipids and their analogs suppress activity of K^+ channels (37).

The observed in the present work changes of the membrane dipole potential are of the order (and even higher) than typical values of the transmembrane potentials, and may probably affect functioning of the voltage-gates ion channels. Moreover, the direction of the change, an increase, creates an additional barrier for cations to penetrate through membrane. It seems therefore plausible that the presence of lidocaine causes blocking of Na^+ ion channels through the change of the electrostatic potential. Due to the difference in the partition coefficients, the uncharged form of lidocaine should be prevailing (at neutral pH) in the membrane interior comparing to the charged form. It is important in this connection that even uncharged lidocaine causes an increase of the potential in the middle of membrane. It is also worth to note, that the observed increase of the electrostatic potential in the presence of uncharged lidocaine takes place mostly due to partial charges associated with the carbonyl group in the middle of the molecule. Such carbonyl group is also present in many other local anesthetics such as tetracaine, procaine, or bupivacaine.

Acknowledgments

This work has been supported by the Swedish Research Council (Vetenskapsrådet). The computing facilities have been granted by the Center for Parallel Computing at the Royal Institute of Technology, Stockholm, and the High-Performance Computer Center at the Umeå University.

References

1. Eckenhoff, R.G. 2001. Promicuous Ligands and Attractive Cavities: How do the inhaled anesthetics Work? *Molecular Interventions* 1: 258-268.

2. Meyer, K.H. 1937. Contribution to the Theory of Narcosis. *Trans Faraday Soc*. 33:1060-1068.

3. Overton, C. E. 1901. Studien Uber die Narkose, *Zugleich ein Beitrag zur Allegemeiner Pharmakologie*. Gustav Fischer, Jena, Switzerland. Translation: *Studies of Narcosis*. 1990. Chapman and Hall. London.

4. Hille, B., 1977. Local anesthetics: Hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J.Gen.Physiology* 69: 497-515

5. Nilius, B., K. Benndorf, and F. Markwardt. 1987. Effects of lidocaine on single cardiac sodium-channels, *J. Mol. and Cellular Cardiol.* 19: 865-874.

6. Butterworth, J.F., and G.R. Strichartz. 1990. Molecular mechanisms of local anesthesia: a review. *Anesthesiology* 72: 711-734.

7. Weiser, T. 2006. Comparison of the effect of four Na^+ channel analgesics on TTX-resistant Na^+ currents in rat sensory neurons and recombinant Mav 1.2 channels, *Neuroscience Lett.* 395: 179-184

8. Narahashi, T., D.T. Frazier, and M. Yamada. 1969. Cationic forms of local anesthetics block action potentials from inside nerve membrane. *Nature* 223: 748-749.

9. Franks, N.P., and W.R. Lieb. 1994. Molecular and cellular mechanisms of general anestesia. *Nature*. 367:607-614.

10. Sheets, M.F., and D.A. Hanck. 2003. Molecular action of lidocaine on the voltage sensors of sodium channels, *J.Gen.Physiology,* 121: 163-175

11. Lipkind, G.M., and H.A. Fozzard. 2005. Molecular modeling of local anesthetic drug binding by voltage-gated sodium channels, *Mol. Pharmacology*, 68 : 1611-1622

12. Ragdale, D.S., McPhee, J.C., Scheuer, T., and W:A. Catterall. 1994. Molecular determinants of state-dependent block of Na+ channels by local anesthetics. *Science* 265: 1724-1728

13. Cafiso, D.S. 1998. Dipole potentials and spontaneous curvature: membrane properties that could mediate anesthesia. *Toxicology Letters*. 100-101: 431-439.

14. Ueda, I., and T. Yoshida. 1999. Hydration of lipid membranes and the action mechanisms of anesthetics and alcohols, *Chem. and Phys. of Lipids* 101: 65 - 79.

15. Cantor, R.S.. 1999. The influence of membrane lateral pressures on simple geometric models of protein conformational equlibria. *Chemistry and Physics of Lipids*. 101: 45-56.

16. da Neto, M.J.D., and R.B. de Alencastro. 1997. Theoretical studies on local anesthetics: Procaine, Lidocaine, Tetracaine, Bupivacaine, and Dibucaine - Neutral and Monoprotonated. I*nt.J.Quant.Chem*., 61: 959-980.

17. Avdeef, A., K.J. Box, J.E.A. Comer, C. Hibbert, and K.Y. Tam. 1998. pH-metric logP10. Determination of liposomal membrane-water partition coefficients of ionizable drugs. *Pharmaceutical Research*, 15 : 209-215.

18. Matsuki, H., M. Yamanaka, H. Kamaya, S. Kaneshina, and I. Ueda. 2005. Dissociation equilibrium between uncharged and charged local anesthetic lidocaine in a surface-adsorbed film, *Colloid Polym. Sci.*, 283: 512-520.

19. Pastor, R.W. 1994. Computer simulations of lipid bilayers, *Current Opinion in Struct. Biol.* 4: 486-492

20. Tieleman, D.P., and H.J.C. Berendsen. 1996. Molecular dynamics simulations of a fully hydrated dipalmitoylphosphatidylcholine bilayer with different macroscopic boundary conditions and parameters. *J.Chem.Phys.* 105: 4871-4880

21. Berger, O., O. Edholm, and F. Jahnig. 1997. Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure and constant temperature, *Biophys. J.* 72: 2002-2013

22. Scott, H.L. 2002. Modeling the lipid component of membranes. *Current Opinion in Struct. Biol*. 12: 495-502

23. Lopes, F.L., S.O. Nielsen, and M.L. Klein. 2004. Hydrogen bonding structure and dynamics of water at the dipalmitoylphosphatidylcholine lipid bilayer surface from a molecular dynamics simulation. *J.Phys.Chem.B* 108: 6603-6610

24. Högberg, C.J., and A.P. Lyubartsev. 2006. A molecular dynamics investigation of the influence of hydration and temperature on structural and dynamical properties of a dimyristoylphosphatidylcholine bilayer, *J.Phys.Chem.B* 110: 14326-14336.

25. Falck, E., M. Patra, M. Karttunen, M.T. Hyvönen, and I. Vattulainen. 2004. Lessons of slicing membranes: Interlplay of packing, free area, and lateral duffusion in phospholipid/cholesterol bilayers *Biophys.J.* 87: 1076-1091

26. Patra, M., E. Salonen, E. Terama, I. Vattulainen, R. Faller, B.W. Lee, J. Holopainen, and M. Karttunen. 2006. Under the influence of alcohol: The effect of ethanol and methanol on lipid bilayers, *Biophys.J.* 90: 1121-1135

27. Pedersen, U.R., G.H. Peters, and P. Westh. 2007. Molecular packing in 1-hexanol-DMPC bilayers studied by molecular dynamics simulations. *Biophys. Chem*. 125: 104-111.

28. Högberg, C.J., A. Maliniak, and A.P. Lyubartsev. 2007. Dynamical and Structural Properties of Charged and Uncharged Lidocaine in a Lipid Bilayer. *Biophys. Chem*., 125: 416-424.

29. Lindahl, E., B. Hess, and D. van der Spoel. 2001. GROMACS 3.0: A package for molecular simulations and trajectory analysis. *J. Molec. Mod*., 7 : 306-317.

30. Darden, T.A., D. York, and L. Pedersen. 1993. Particle mesh Ewald: An *NlogN* method for Ewald sums in large systems. *J. Chem. Phys.*, 98: 10089-10092.

31. Wohlert, J., and O. Edholm. 2004. The range and shielding of dipole-dipole interactions in phospholipid bilayers. *Biophys. J*. 87: 2433-2445.

32. Cevc, G. 1990. Membrane Electrostatics. *Biochem.Biophys.Acta.* 1031: 311-382.

33. Clarke, R.J. 2001. The dipole potential of phospholipid membranes and methods for its detection. *Adv. Colloid and Interface Sci.* 89-90: 263-281.

34. Wang L., P.S. Bose, and F.J. Sigworth, 2006. Using cryo-EM to measure the dipole potential of a lipid membrane. *Proc. Natl. Acad. Sci. USA* 103:18528-18533.

35. Seelig J., P.M. Macdonald, and P.G.Scherer, 1987. Phospholipid head group as sensors of electric charge in membranes. *Biochemistry* 26: 7535-7541.

36. Gurtovenko A.A., M. Patra, M. Karttunen and I. Vattulainen, 2004. Cationic DMPC/DMPTAP Lipid bilayers: Molecular Dynamics Study, *Biophys J.* 86: 3461-3472

37. Pertou, S., R.W.Ordway, J.A.Hamilton, J.V. Walsh, Jr, and J. J. Singer, 1994. Structural requirements for charged lipid molecules to directly increase or suppress K+ channel activity in smooth muscle cells., *J. Gen. Physiol.* 103: 471-486.

Table 1. Some properties of the simulated systems. Φ(0) is the electrostatic potential in the middle of membrane while Φ_{max} is its maximal value. For other details, see the text.

Simulated System	**Area per Lipid** (Å^2)	**Φ(0)** (V)	**Φ_{max}** (V)	**Headgroup Angle** (degrees)
Reference	64.2	0.560	0.76	79.8
12 Lidocaine	65.4	0.656	0.74	79.0
12 Charged Lidocaine	64.4	0.651	0.80	72.2
36 Lidocaine	67.6	0.770	0.73	79.0
36 Charged Lidocaine	66.8	0.781	0.84	60.
uncertainty	0.15	0.002	0.01	0.5

Figures.

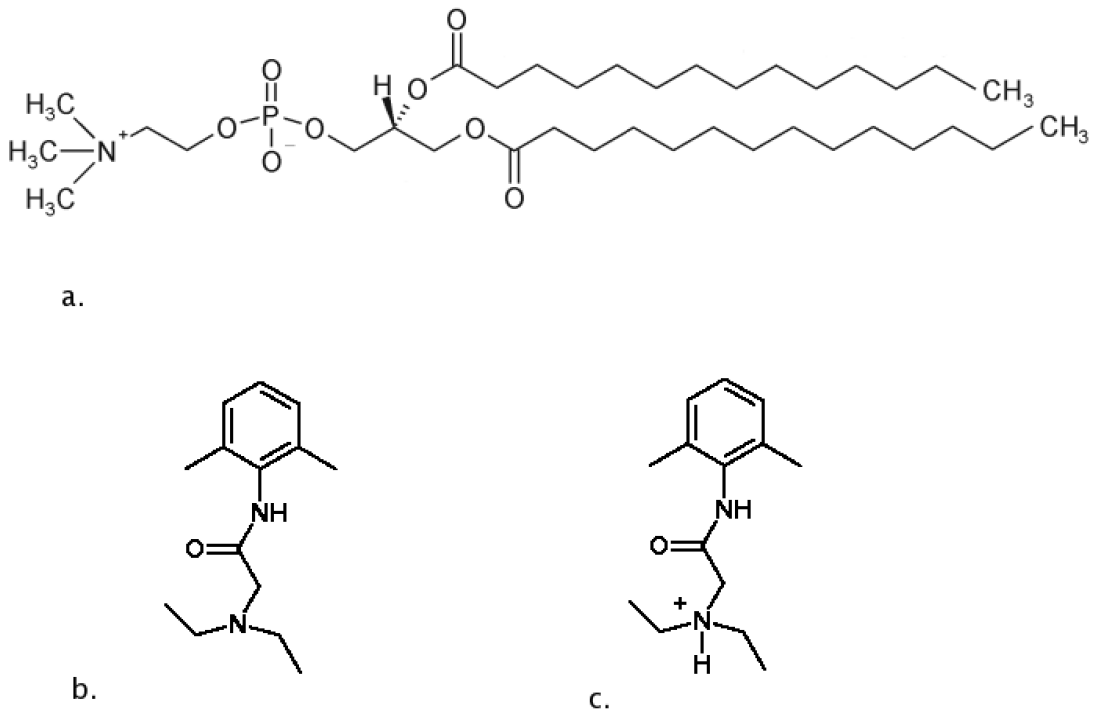


Figure 1. Molecular structures used in the simulations. (a) DMPC, (b) uncharged lidocaine (c) charged lidocaine.

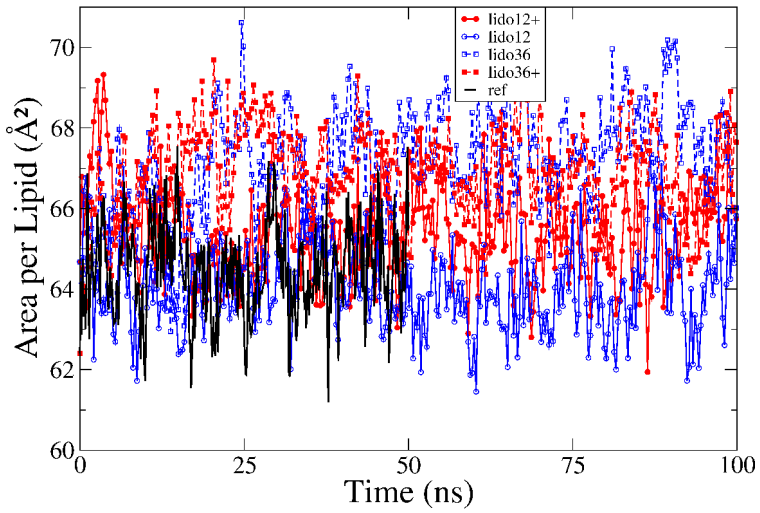


Figure 2. Evolution of the area per lipid. Charged lidocaine: red lines with filled symbols, uncharged: blue lines with open symbols; 12 lidocaine: solid lines with circles; 36 lidocaine: dashed lines with squares; reference system: black line without symbols.

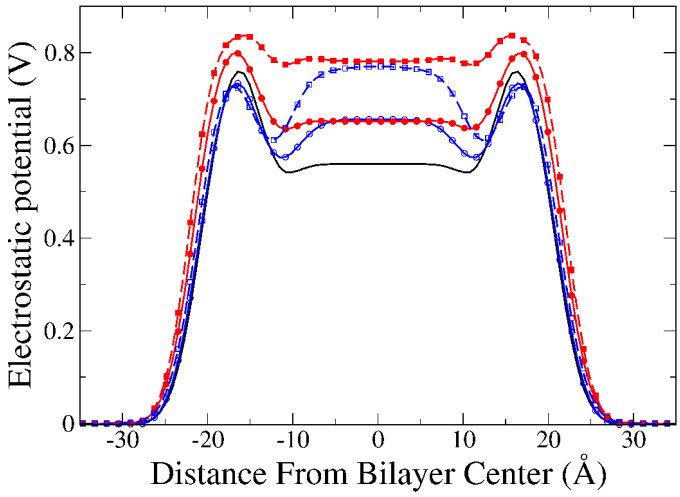
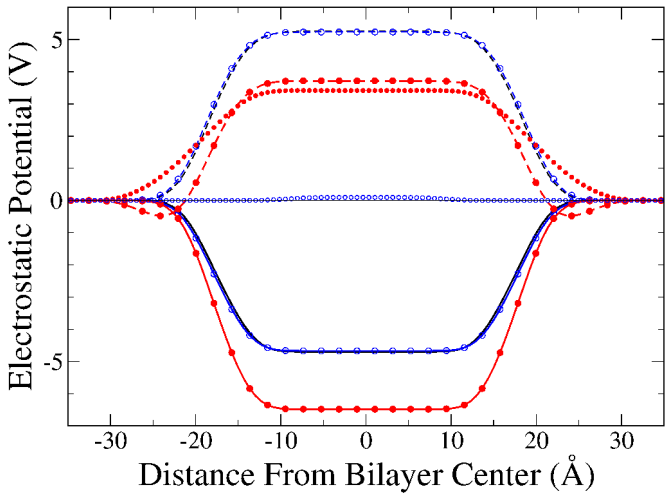


Figure 3. The electrostatic potential. Charged lidocaine: red lines with filled symbols, uncharged: blue lines with open symbols; 12 lidocaine: solid lines with circles; 36 lidocaine: dashed lines with squares; reference system: black line without symbols.

a



b

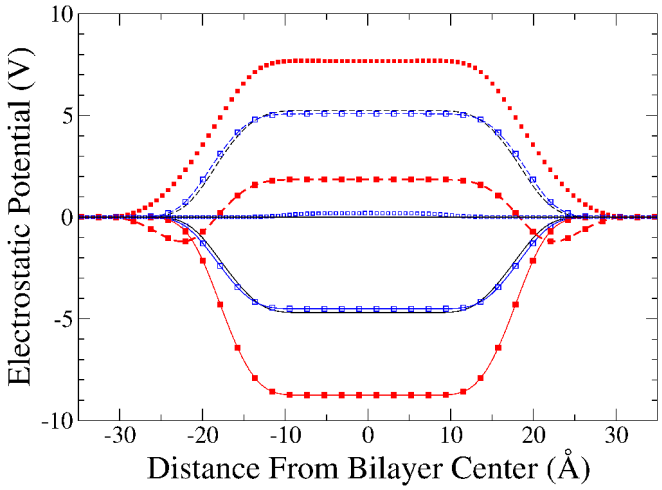


Figure 4. Different contributions to the electrostatic potential. (a) 12 lidocaine molecules; (b): 36 lidocaine molecules. DMPC: solid lines, water: dashed lines, lidocaine (with Cl^- ions if charged): symbols without line. Uncharged systems: blue lines with open symbols; charged systems: red lines with filled symbols; reference system: black lines without symbols.

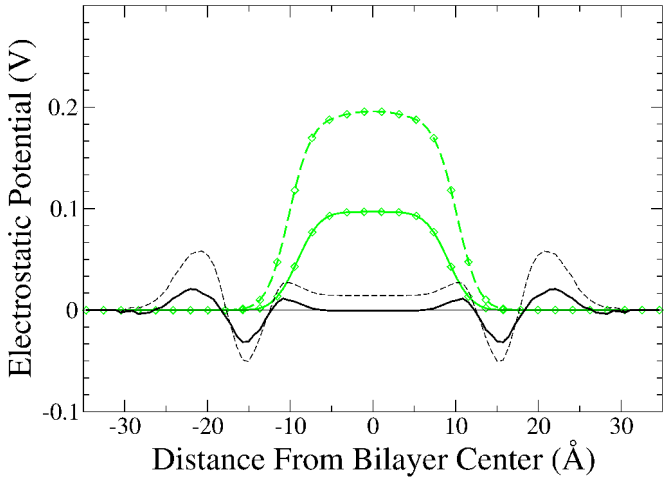


Figure 5. Contribution of the uncharged lidocaine to the total electrostatic potential (green lines with diamonds) and contribution of water and lipids to the change of the electrostatic potential upon addition of lidocaine (black lines without symbols). 12 lidocaine: solid lines, 36 lidocaine: dashed lines.

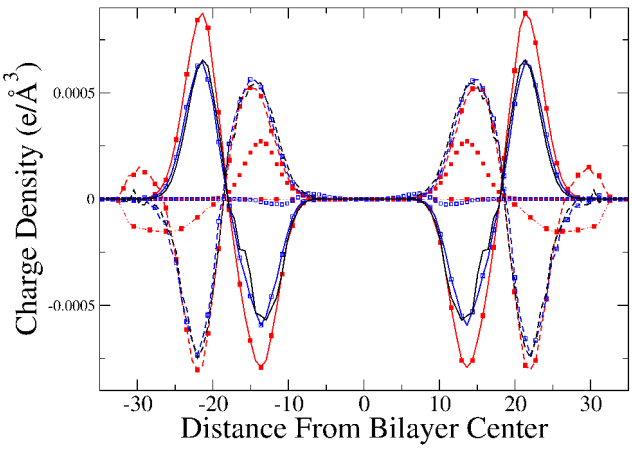


Figure 6. Contributions to the charge density from different components in the systems with 36 lidocaine and in the reference system. DMPC: solid lines, water: dashed lines, lidocaine dots, Cl- ions: dot-dashed line. Uncharged systems: blue lines with open symbols; charged systems: red lines with filled symbols; reference system: black lines without symbols.

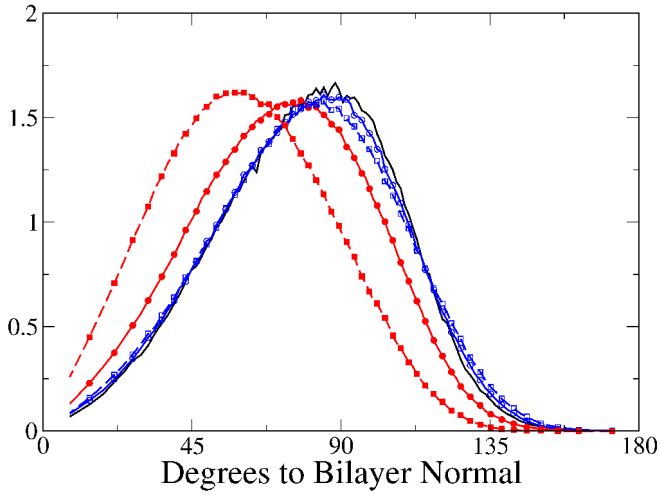


Figure 7. Distribution of the angle between phosphorus to nitrogen vector and the bilayer normal for each of the leaflets, the normal is directed out of the bilayer. Charged lidocaine: red lines with filled symbols, uncharged: blue lines with open symbols; 12 lidocaine: solid lines with circles; 36 lidocaine: dashed lines with squares; reference system: black line without symbols.