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Calcium and protons affect the interaction of neurotransmitters and anesthetics with anionic lipid membranes

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ABSTRACT

We study how zwitterionic and anionic biomembrane models interact with neurotransmitters (NTs) and anesthetics (ATs) in the presence of Ca^{2+} and different pH conditions. As NTs we used acetylcholine (ACh), γ-aminobutyric acid (GABA), and L-glutamic acid (LGlu). As ATs, tetracaine (TC), and pentobarbital (PB) were employed. By using differential scanning calorimetry (DSC), we analyzed the changes such molecules produce in the thermal properties of the membranes. We found that calcium and pH play important roles in the interactions of NTs and ATs with the anionic lipid membranes. Changes in pH promote deprotonation of the phosphate groups in anionic phospholipids inducing electrostatic interactions between them and NTs; but if Ca^{2+} ions are in the system, these act as bridges. Such interactions impact the physical properties of the membranes in a similar manner that anesthetics do. Beyond the usual biochemical approach, we claim that these effects should be taken into account to understand the excitatory-inhibitory orchestrated balance in the nervous system.

1. Introduction

Interneuronal communication through synapses shares nearly the same molecular mechanism involved in the anesthesia phenomenon: chemical or electrical stimuli produce conformational changes in proteins to enhance or decrease the transmission of a given signal [1–3].

Such electrophysiological events, that ultimately yield nervous transmission [4,5], are induced by molecules known as neurotransmitters and can be modified by the anesthetic action. Neurotransmitters and anesthetics are molecularly diverse. Small molecules (acetylcholine, adenosine, anandamide), monoamines (dopamine, norepinephrine, epinephrine, serotonin and melatonin), amino acids (glutamate, aspartate, serine, γ-aminobutyric acid), and short peptides are important neurotransmitters [6,7]; whereas alkanes, alcohols, ethers, esters, amides, phenols, benzodiacepines, barbiturates, and even inert gases are anesthetics [8].

Synaptic transmission takes place when an electric pulse (action potential) is transmitted by neurotransmitters from presynaptic to postsynaptic terminals. Though this mechanism has been vastly investigated, essential questions like the role of calcium and pH at synapses remain puzzling [9–15].

In the synaptic process, calcium acts as a triggering agent: in exocytosis fusion and the subsequent NTs release; as a second messenger to start signalling cascades; or as a metabolite to be sensed by proteins [16–19]. Although this divalent atom plays an important role to release and uptake neurotransmitters mediated by SNAREs (soluble NSF (*N*-ethylmaleimide) attachment protein receptor) and synaptotagmins, the orchestrated docking, zippering, triggering, fusion, recycling interneuron mechanism is not well understood. [12–14,19]. Similarly, although protons have been proposed as neurotransmitters [15], their levels and fluctuations at synapses give rise to electrostatic effects on the membrane no completely known.

Anesthesia, on the other hand, is the process whereby the communication between neurons is disturbed. Two mechanisms have been proposed to explain it: the first one is unspecific (the action is on lipids), the second one specific and the targets are proteins [1,20–23]. Despite the overwhelming endorsement of the second option, some reports support the idea that entropic changes in lipid rafts, where proteins seem to anchor, may drive anesthetic action [24]. Therefore, the crucial role of lipids in neural structure and function demands that we maintain them as viable subjects of further research [25].

Heimburg et al. developed a thermodynamic theory about general and local anesthesia, where they proposed a daring model about the nervous impulse. They consider that this has its origin in the changes

Abbreviations: Cp, specific heat capacity at constant pressure; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (sodium salt); DPPG, 1,2-dihexadecanoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt); DMPS, 1,2-ditetradecanoyl-*sn*-glycero-3-phospho-L-serine (sodium salt); SM, *N*-octadecanoyl-D-*erythro*-sphingosylphosphorylcholine; ACh, acetylcholine; GABA, γ-aminobutyric acid; LGlu, L-glutamic acid; TC, tetracaine; PB, pentobarbital; SUV, sonicated unilamellar vesicles; T_m, phase transition temperature.

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of the elastic and thermodynamic properties of the membrane [23,26–28]. Moreover, Cantor suggests that changes in the lateral pressure of the membrane produced by anesthetics trigger receptor desensitization [29]. He also discusses how neurotransmitters, seen as endogenous anesthetics in living organisms, diffuse into the postsynaptic membrane affecting their physical properties by promoting a conformational disequilibrium of receptors, i.e. desensitization [30]. This hypothesis has been tested more recently by Jerabek et al. by using X-ray diffraction and molecular dynamic simulations [31].

The idea that the plasma membrane is relevant in neural transmission is very plausible for the following reason: it has domains with negative charges due to the prevalence of anionic phospholipids [32–34] that regulate diverse physiological processes through electrostatic interactions [33,35–40], in which the phosphatidic acid plays an important role in the signalling events [41].

Pure lipid membranes are well established biological models to study mechanical, thermodynamic and electrostatic properties associated with real biological membranes [42–44]. For instance, the phase transition temperature (T_m), at which the ordered phase($L_{\beta'}$) changes to the fluid phase (L_α), is a property vastly studied in membrane models to depict functional processes of the cell [45]. Beyond the accepted model where NTs are seen as specific ligands, unspecific NTs-bilayer binding mechanisms are currently emerging. By using atomistic molecular dynamics simulations, Orlowski et al. observed strong binding of dopamine and L-dopa with bilayers containing anionic lipids (phosphatidylserine), sphingomieline, and cholesterol. They conclude that these interactions depend on the H-bonds and charge pairs with the polar groups of the lipids [6]. They also estimated the surface pressure changes of the DOPC-DOPE-DOPS monolayers. In addition, using calorimetry tools, Piorecka et al. reported the first experimental evidences about the preponderant role of negative charge in the interaction of dopamine with an anionic biomembrane model (DMPC/DMPG) [46], concluding that hydrophobic forces are weaker than electrostatic ones. Furthermore, Drolle et al. carried out small-angle neutron scattering and diffraction experiments, together with molecular dynamics simulations, to demonstrate that melatonin (another neurotransmitter) fluidizes DPPC and DOPC bilayers, decreasing their thickness with an increment of the head group area [47]. Choi et al. confirmed the disorder of the lipid membranes caused by melatonin using a Langmuir-Blodgett film technique. They showed that the hydrophobic interactions are the main way melatonin interacts with cholesterol and phospholipids without a specific target [48]. Recently, essential clues about the interplay between NTs and a lipid membrane were reported (again using molecular dynamics simulations), demonstrating that NTs do not permeate into membranes but show only reversible adhesion [49]. The authors claim that if the receptor is buried in the lipid bilayer, the interaction is membrane-dependent where anionic lipids and ions are crucial. Interestingly, acetylcholine follows such proposed mechanism.

In a similar fashion, the effect produced by monovalent and divalents cations in lipid membranes have been investigated experimentally. Divalent cations (e.g., Ca^{2+}, Mg^{2+}) can interact strongly with both zwitterionic and anionic lipids [50,51], but the interactions with anionic lipids (e.g., DMPS, DPPA) is rather remarkable [50], resulting in cochleation events governed by dehydration and chelation of head-group lipids [50,52]. In contrast, although monovalent cations (i.e., Na^+) penetrate deeply into a membrane and bind specifically to carbonyl oxygens of phospholipids, they slightly affect the physical properties of DOPC, even at high concentrations [53].

In this context, we aim to combine all these efforts in a broader picture to understand how neurotransmitters and anesthetics interact with lipid (zwitterionic and anionic) bilayers in the presence of calcium and different pH conditions. Our results teach us that neurotransmitters and anesthetics can produce similar effects in lipid bilayers: depression of the transition temperature T_m. Moreover, we find that Ca^{2+} and H^+ are preponderant players in these interactions.

2. Methods

2.1. Reagents

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (sodium salt), 1,2-dihexadecanoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt), 1,2-ditetradecanoyl-*sn*-glycero-3-phospho-L-serine (sodium salt) and *N*-octadecanoyl-D-*erythro*-sphingosylphosphorylcholine (brain porcine) were purchased from Avanti Polar Lipids, Birmingham, AL. Calcium chloride, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, 2-amino-2-methyl-1,3-propanediol, citric acid, trisodium citrate dihydrate, acetylcholine chloride, γ-aminobutyric acid, L-glutamic acid (monosodium salt monohydrate) and tetracaine hydrochloride were purchased from Sigma-Aldrich. Pentobarbital (sodium salt) was purchased from Cheminova. All chemistry reagents were used without further purification.

2.2. Liposomes and sample preparations

Multilamellar vesicles (MLV) were generated using a thin film of a suspension made of DPPC:SM, DPPC:DPPA, DPPC:DPPG or DPPC:DMPS. To prepare the DPPC:SM vesicles, DPPC and SM were dissolved in chloroform. For the three others, each lipid was dissolved in a mixture of methanol and chloroform 1:1 (v/v). The organic solutions were softly vortexed to homogenize them, and the solvents were evaporated by rotatory evaporation. Then, the lipid films were kept in a vacuum chamber for 3 h to eliminate solvent traces. The films were hydrated with the following solutions: pH 5.0, citric acid-sodium citrate buffer 10 mM; pH 7.4, HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) 10 mM; pH 9.0, AMPD (2-amino-2-methyl-1.3-propanediol) 10 mM; uncontrolled pH (4–6), Milli-Q-water, 18.2 M V cm at 60 °C. For all the suspensions we apply stirring at 1000 rpm for 60 min. For experiments with calcium, the films were hydrated with a $CaCl_2$ solution (10 mM, or 2 mM, previously dissolved in the buffer solution or water) at 7 °C, 1000 rpm for 60 min.

To prepare small unilamellar vesicles (SUV), 0.820 ml of the MLV suspension were sonicated for 30 min at 50 °C in a water-bath ultrasonicator (60 W). Thereafter, a solution with neurotransmitters or anesthetics was added to the SUV dispersion at 25 °C, homogenized by vortexing for 1 min and degassed in a Degassin Station Model 6326 (TA Instruments) for 10 min at 600 mm Hg at 25°C while stirring at 400 rpm. The final concentrations used in each experiment were 5 mM of lipid mixture (at 1:1 M ratio), and 25 mM for neurotransmitters and anesthetics.

2.3. Calorimetric analysis

Calorimetric measurements were done in a microcalorimeter NanoDSC (TA Instruments). Heat capacity profiles were recorded at a constant pressure (3 atm) and constant scan rate of 1 °C/min (the high-sensitivity power-compensation of the instrument allows us to use fast scan rates) [54,55]. After loading the samples into the DSC cell and before the scans started, these were equilibrated for 5 min. Each sample was scanned in a heating mode three times and, due to the high reproducibility, the measurements were repeated only two times. To acquire the data, the calorimeter was interfaced into a PC. Data were analyzed using the software provided with the equipment.

3. Results and discussion

3.1. Ca^{2+}, pH, and neurotransmitters change the thermal properties of anionic membranes

While Ca^{2+} and localized proton fluctuations in the synaptic clefts are relevant to the orchestrated process of neural transmissions, there are still many unanswered questions regarding the specific role they

play [15]. For instance, little has been explored about the thermodynamic changes in synaptic terminals due to the influence of such ions. To assess these aspects we measure calorimetric profiles of lipid membranes in the presence of three neurotransmitters, ACh, GABA, and LGlu with different ion conditions.

Moreover, to mimic the electrostatic aspects of real membranes, several lipids are used: zwitterionics (DPPC and SM) and anionics (DPPA, DPPG, DMPS). We explored four different combinations: DPPC:SM, DPPC:DPPA, DPPC:DPPG and DPPC:DMPS; all of them at 1:1 M ratios. As previously mentioned, the concentration employed for the neurotransmitters was 25 mM. We would like to comment that despite this concentration could be considered quite high, the fact is it is not. Concentrations inside carrying vesicles are around 250 mM [30], warranting transients of high concentrations liberated in the synaptic clefts [56].

Fig. 1 shows the calorimetric profiles of DPPC:SM and DPPC:DPPA vesicles interacting with ACh and LGlu (excitatory neurotransmitters) and GABA (an inhibitory neurotransmitter). In the first case, we evaluated the effects of Ca^{2+} (10 mM) at pH 7.4. In the second case, for the anionic vesicles, we measured such effects at fluctuating pH conditions that mimic the real situation in synaptic processes [15].

Firstly, the zwitterionic membranes do not show any relevant interaction with the three neurotransmitters. Indeed, all the transition temperatures are the same as the T_m of the control, which is 38.3 °C. The transition enthalpies (ΔH) are around 40 kJ/mol (see Supplementary Fig. S1). These values were obtained regardless if calcium is present or not. We must remark that infrared spectroscopy has revealed the existence of calcium binding with a zwitterionic phospholipid, involving the carbonyl and phosphodiester groups [51]; however, for low molecular cooperativities (obtained when two or more lipids are used to form the bilayer), such binding is non-existent or rather small [57].

Secondly, in the case of the DPPC:DPPA vesicles at buffer conditions (pH 7.4), ACh and LGlu substantially change the calorimetric profiles. As a matter of fact, while the T_m and ΔH of the pure membranes are 58.1 ± 0.6°C and ΔH 30.4 ± 2.2 kJ/mol (see Supplementary Fig. S1), respectively, upon the addition of the neurotransmitters they change: ACh reduces T_m and ΔH to 54.9 ± 0.5° and 29.4 ± 1.2 kJ/mol, respectively, and LGlu does it to 54.5 ± 0.6°C and 23.7 ± 2.4 kJ/mol. Note that GABA does not alter T_m at all, although ΔH is reduced to 23.1 ± 1.1 kJ/mol. Calcium promotes a substantial influence in the lipid cooperativity, remarkably reducing T_m and thus the cohesion of the vesicles. Furthermore, three peaks show up in the heat capacity profile of the membrane, suggesting the coexistence of three domains produced by the separation and aggregation of vesicles induced by calcium as reported decades ago [50]: i) membranes plenty of DPPC (whose transition is around 43 °C); ii) a mixture of DPPC:DPPA near to 46 °C; and iii) membranes where DPPA is dominant, around 52 °C. In each case, the total ΔH is 20 kJ/mol. Note that GABA and LGlu debilitate such domains.

If the electrostatic effect produced by calcium is already patent in the thermometric signatures of the studied vesicles (central panels of Fig. 1), the screening of the polar lipid heads is even more notorious when pH is not buffered. Indeed, the calorimetric profiles of the anionic DPPC:DPPA membranes with all three neurotransmitters are different (upper right panel of Fig. 1). If Ca^{2+} is now added to the medium, the fusion of membranes is arrested and the effect of neurotransmitters is even more stronger depending on which one is employed; LGlu and GABA being the more influencing molecules in the loss of cooperative strength (see lower right panel of Fig. 1).

Overall, calcium and unbuffered pH influence the cooperativity of vesicles and the formation of lipid domains, suggesting the relevant role of electrostatics in modifying the surface pressure, thickness and curvature of the membrane [58]. Our results suggest that neurotransmitters interact with anionic lipid membranes through an electrostatic mechanism [59,60] mediated by Ca^{2+} and H^+. ACh, in the absence of calcium lessens the cohesion of the membrane, but this effect is reversed when calcium and protons are in the medium (due to a competitive mechanism between Ca^{2+} and ACh, which has a positive charge on the trimethylammonium cation, or due to an electrostatic repulsion between the DPPA-Ca complex and ACh). On the contrary, GABA lessens the cohesion with calcium, likely by interactions of ions and counterions similarly to a model of electrical double layer, [61] where Ca^{2+} acts as a zippering between the negative surface of the membrane and the carboxylate group of GABA (which is a zwitterionic molecule). This behavior may be related with the excitatory effect of ACh and the inhibitory effect of GABA in the neural system [7]. LGlu interacts with lipid membranes stronger than GABA because LGlu has two carboxylate groups.

To evaluate the influence of calcium and protons at physiologically relevant concentrations, and taking into account the chemical nature of the charges composing the membrane, we measured calorimetric profiles of DPPC:DPPA, DPPC:DPPG, DPPC:DMPS with ACh, GABA, and LGlu. Despite these lipids are amphiphilic, the hydrophilic heads have different compositions: DPPC has a choline group bonded to a phosphate; DPPA has a phosphatidic acid, DPPG bonds to second glycerol group, and DMPS has a serine group bonded to phosphate.

Fig. 2 compares the DSC analysis of such anionic membranes. The curves of DPPC:DPPA, with 2 mM of calcium at a pH of 7.4, indicate the tendency towards the formation of domains, similarly to the results obtained at a higher concentration (10 mM of calcium). However, at 2 mM the effect of ACh, GABA, and LGlu are less noticeable (see

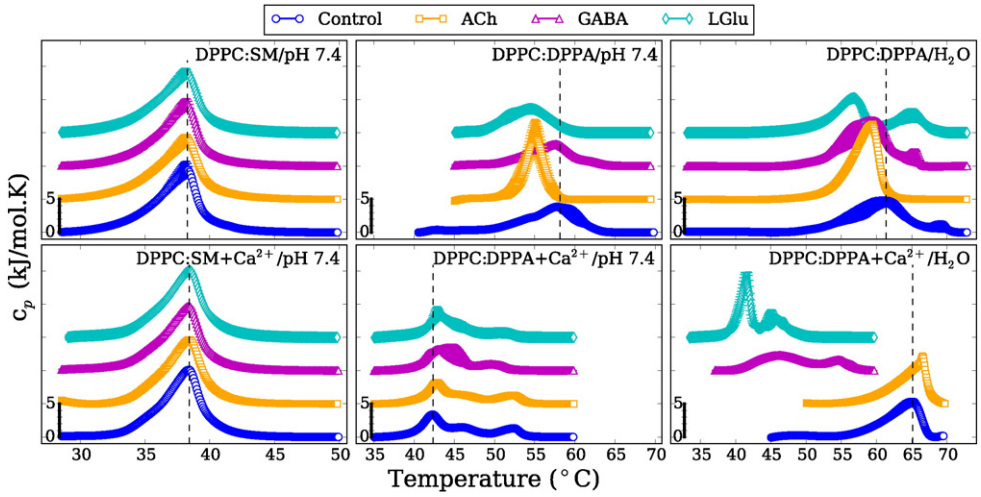

Fig. 1. Calorimetric profiles of zwitterionic SUVs of DPPC:SM (1:1 M ratio) and anionic SUVs of DPPC:DPPA (1:1 M ratio), and their interactions with three neurotransmitters, ACh (25 mM), GABA (25 mM) and LGlu (25 mM), in the absence and presence of Ca^{2+} (10 mM). DPPC:DPPA experiments were developed at pH 7.4 (10 mM HEPES) and under H^+ fluctuations (H_2O milliQ). SUVs without neurotransmitters were used as controls (dashed line marks main transition of controls).

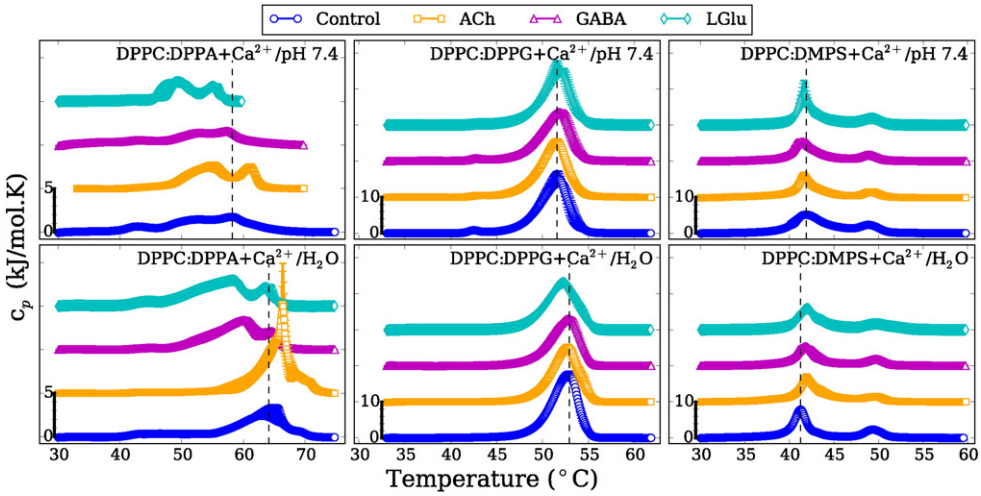

Fig. 2. Calorimetric profiles of anionic SUVs at 1:1 M ratio of DPPC:DPPA, DPPC:DPPG, and DPPC:DMPS. Interacting with ACh (25 mM), GABA (25 mM) and LGlu (25 mM), under the influence of 2 mM Ca^{2+}. All experiments were developed at pH 7.4 (10 mM HEPES) and under H^+ fluctuations (H_2O milliQ). SUVs without neurotransmitters were used as controls (dashed line marks main transition of controls).

Supplementary Fig. S2). At these conditions, the control has three peaks in the heat capacity. While the calorimetric profile of the vesicles with GABA is similar to the profile of the control (with three peaks and 22.5 ± 0.8 kJ/mol for ΔH), membranes with ACh only display two transitions (at 54.6 ± 0.8°C and 61.2 ± 0.2 °C). Membranes with LGlu displays also two peaks, but these are shifted to lower temperatures and the total enthalpy is lower. In contrast, when pH fluctuates, the T_m for DPPC:DPPA/Ca/neurotransmitter shifts to higher temperatures. Vesicles with GABA or LGlu exhibit also two transitions.

DPPC:DPPG vesicles with neurotransmitters display higher cooperativity in both conditions, pH 7.4 and unbuffered pH, but the T_m in the first case has a lower value than the second one. The calorimetric profiles obtained for the third anionic system (DPPC:DMPS) show again two transitions, due to the presence of domains. However, the transition temperatures are all similar.

The classical mechanism of transmission at chemical synapses states that NTs reach their respective binding sites by a random diffusion in the bulk water without adhering to the lipid membrane. But in this environment all components are subject to non-specific interactions. Indeed, Wang et al. analyzed the affinity of neurotransmitters with a pure lipid membrane model [62]. They found, by dialysis in equilibrium, that neurotransmitters interact weakly with anionic membranes. They suggest that coulombic forces and specific polar interactions govern neurotransmitter binding. Also, Peters et al. performed molecular dynamic simulations to study the effect of serotonin with a lipid membrane [63]. In addition, atomistic molecular dynamic simulations have recently revealed that NTs aggregate on the membrane before diffusing to reach buried binding sites [49]. Such process is influenced by anionic lipids, which promote the partitioning of NTs. All in all, our results seem to be in agreement with the idea that anionic membranes play an important role during the interaction with neurotransmitters, highlighting the phosphatidic acid as the electrostatic sensor. In this interplay, calcium and protons moderate such interactions.

3.2. Ca^{2+} and pH modify the effects that tetracaine and pentobarbital produce on lipid membranes

Fig. 3 shows calorimetric scans of DPPC:SM and DPPC:DPPA membranes under the action of tetracaine and pentobarbital with and without calcium. In the DPPC:SM case, we measured the heat capacity at a pH of 7.4; in the DPPC:DPPA case, the value of pH was not controlled. Clearly, calcium and protons influence much more the thermal behavior of the anionic membranes in the presence of anesthetics (see also Supplementary Fig. S3). We have mentioned before that neurotransmitters

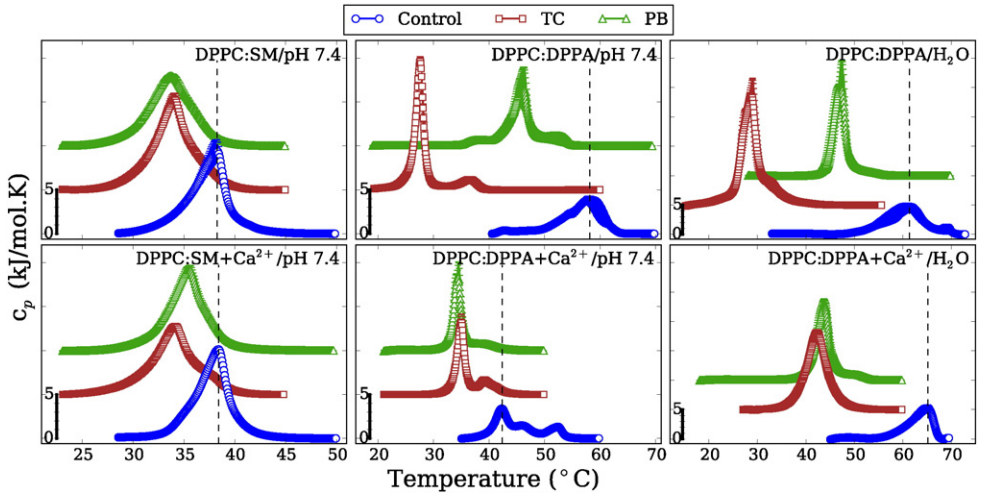

Fig. 3. DSC profiles of DPPC:SM and DPPC:DPPA SUVs (1:1 M ratio), and the effects produced by two anesthetics: TC (25 mM) and PB (25 mM), in the absence and presence of Ca^{2+} (10 mM). DPPC:DPPA experiments were developed at pH 7.4 (10 mM HEPES) and under H^+ fluctuations (H_2O milliQ). SUVs without anesthetics were used as controls (dashed lines show main transitions).

have been catalogued as endogenous anesthetics in living systems [30], and it is a well known fact that anesthetics depress transition phases of lipid membranes [64–69]. Therefore, our results imply the existence of such similarity: depending on the calcium and proton conditions, neurotransmitters produce in lipid membranes the same effect anesthetics do.

Similarly to what we did with neurotransmitters (see Fig. 2), we obtained now calorimetric profiles of three anionic membranes (DPPC:DPPA, DPPC:DPPG, and DPPC:DMPS) interacting with TC and PB under the influence of calcium (2 mM) and unbuffered pH (see Figs. 4 and S4). In a first glance, we observe a clear feature: TC and PB depress T_m irrespectively of the type of vesicle. However, in the DPPC:DPPG case, TC produces a larger shift of the main transition in both environments, at pH 7.4, and uncontrolled pH. This may be explained easily: being TC a weak base, it interacts with the anionic surface of the membrane and thereafter with the aliphatic tails, resulting in a major lowering of T_m than those produced by hydrophobic molecules like PB, which only participates in hydrophobic interactions.

Our previous results demonstrate that calcium and protons mediate the interactions of anionic lipid bilayers with NTs and ATs. All amphoteric molecules have one or several values of pK_a and the proximity of the pH to them determines their equilibrium conditions (where deprotonated and protonated species exist equally). This equilibrium plays a crucial role in the succession of events leading to the interactions we are studying here. To support our findings, where unbuffered conditions change the interaction dynamics of NTs and ATs whether calcium is present or not, we now control the pH below and above 7.4. According to Fig. 5, buffered conditions do affect the calorimetric profiles, indicating that an excess (reduction) of protons blockade (enhance) the effect of calcium. Indeed, in the first case (pH = 5) the membrane remains almost unaltered and the presence of ACh does not change the state. Only the hydrophobic species of TC are able to interact with the bilayer, shifting T_m about 30 °C (see the corresponding panel in Fig. 5). In the case of pH = 9, calcium broadens and flattens the profile indicating the loss of cooperativity. ACh and TC are able to modify this situation: i) ACh recovers the cooperativty due to the fact there is a competitive interaction between the positive charges of calcium and ACh; and ii) TC also recovers the lost of cooperativity because at this pH it is fully hydrophobic, increasing the cohesion of the membrane.

Fig. 6 displays a summary of our results for the interaction of DPPC:DPPA model with neurotransmitters. At the bottom we show the calorimetric behavior induced by ACh, GABA, and LGlu at different conditions of pH and Ca^{2+}. At the top we display the possible molecular mechanism involved: electrostatic interactions among phosphatidic acid, Ca^{2+}, protons, and neurotransmitters. It is known that vesicles formed with DPPC-DPPA (or other anionic lipids) stack when calcium is incorporated to the medium. However, this occurs only when such vesicles are already formed and the concentration of calcium is not in equilibrium (their inner part is free of calcium) producing a destabilization of the membranes [50]. However, when calcium is inside and outside of the vesicles, and therefore in equilibrium (lipids are hydrated when calcium is already in the system, as we do in these experiments), the stacks do not form and we observe low temperature transitions. We suggest that protons interact with the phosphatidic acid that compete in the interaction of DPPA-Ca, which enables the possibility for the neurotransmitters to interact with the system. When there is no buffer to control the pH, one oxygen of the phosphatidic acid is protonated and therefore the interaction occurs only between the deprotonated hydroxyl and GABA or LGlu, with calcium as a bridge. Consequently, the DSC profiles are shifted to lower temperature as the above case. Since ACh is positively charged, the interaction with the phosphatidic acid screened with calcium is now repulsive, there is no cohesive interaction and the profile is almost the same as the control. Similar mechanisms have been previously discussed to explain the interactions between anionic phospholipids, divalent cations and protons [59,60,46,70].

4. Conclusion

We performed a calorimetric study to understand how NTs (ACh, GABA, LGlu) and ATs (TC, PB) affect the thermal properties of zwitterionic and anionic model membranes in the presence of calcium at different pH conditions. Our study has revealed that anionic lipids interact strongly with NTs. Despite the classical mechanism of transmission at chemical synapses states that neurotransmitters interact with specific receptors in the plasma membrane, our results suggest that unspecific electrostatic interactions between neurotransmitters and anionic lipids could induce mechanical and thermal changes in real membranes, resembling the effects that anesthetics produce. These effects can be modified with Ca^{2+} and H^+. Indeed, at different pH conditions the chemical equilibrium of anionic lipids gives either charged or uncharged species, enabling Ca^{2+} to act as a bridge between lipids and neurotransmitters. The thermal results of the excitatory NTs (ACh) demonstrate that this molecule shifts the transition temperature to higher values contrary to the effect observed with anesthetics, which lower the transition temperature. This behavior could be related to the excitatory-inhibitory balance in the neural system. Within this perspective, such mechanical and thermal changes produced in the membrane are important to be

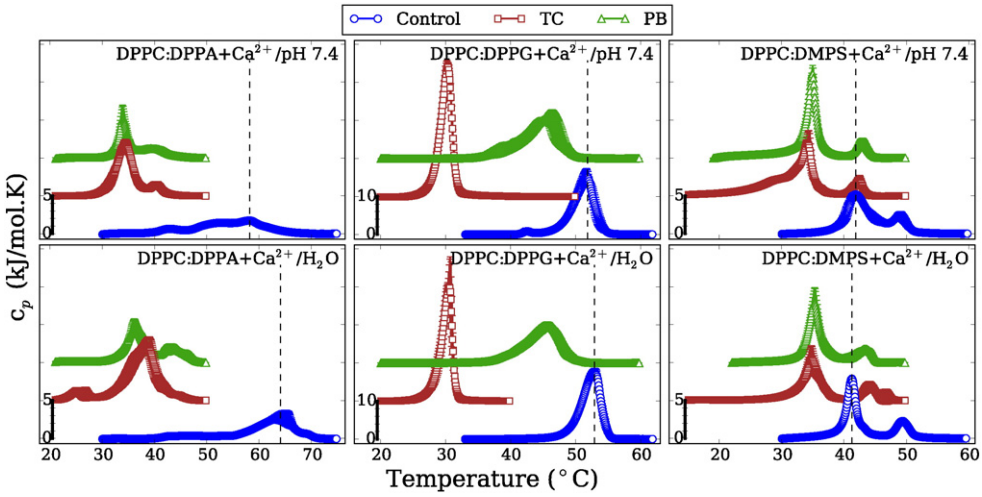

Fig. 4. Calorimetric profiles of the action of TC and PB (both at 25 mM) on anionic DPPC:DPPA, DPPC:DPPG, and DPPC:DMPS SUVs at 1:1 M ratio, under the influence of 2 mM of Ca^{2+}. All experiments were carried out at pH 7.4 (10 mM HEPES) and also under H^+ fluctuation conditions (H_2O milliQ). SUVs with no anesthetics were used as controls (dashed lines mark the temperature transitions).

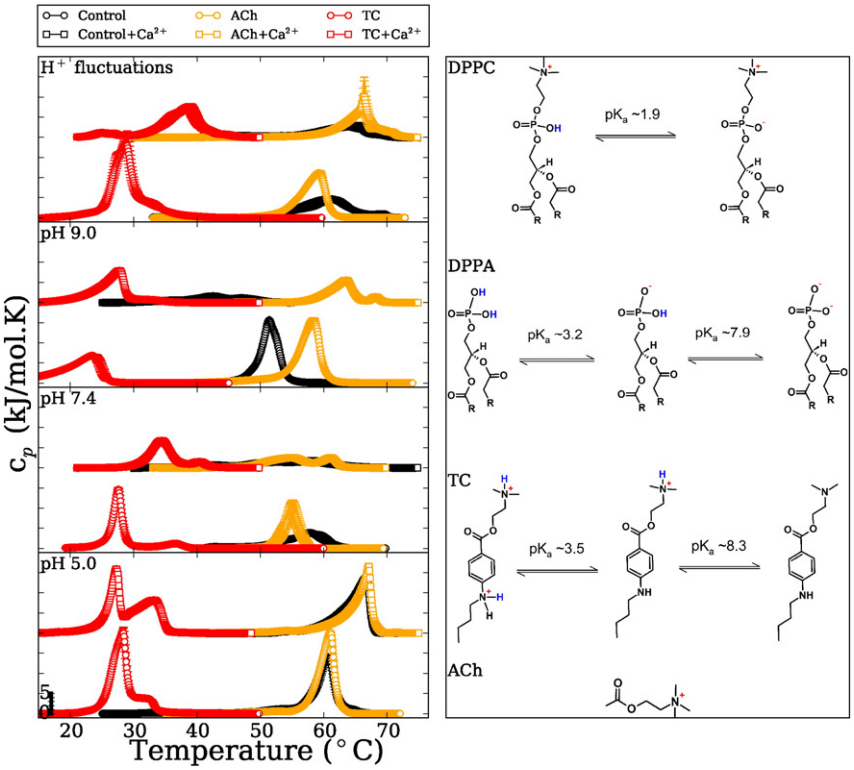

Fig. 5. Changes of calorimetric profiles of the DPPC:DPPA membranes caused by ACh, and TC under different conditions of pH with zero and 2 mM of Ca^{2+}. Equilibrium reactions of DPPC, DPPA, and TC. ACh is not affected by pH.

considered in neural mechanisms. After all, protein receptors are ultimately anchored in lipid membranes.

Now that we have a better picture of the modifications produced by neurotransmitters with calcium and protons in the thermotropic profiles of various anionic vesicles, we would like to measure in a future work partition equilibrium constants. These constants could be important to detail and quantify the interactions under study.

Author contributions

R.P.I. carried out all the experiments and performed the analysis of the data. R.P.I. and J.C.R.S. contributed in the discussion of the results and preparation of the manuscript. J.C.R.S. supervised the research.

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Notes

The authors declare no competing financial interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

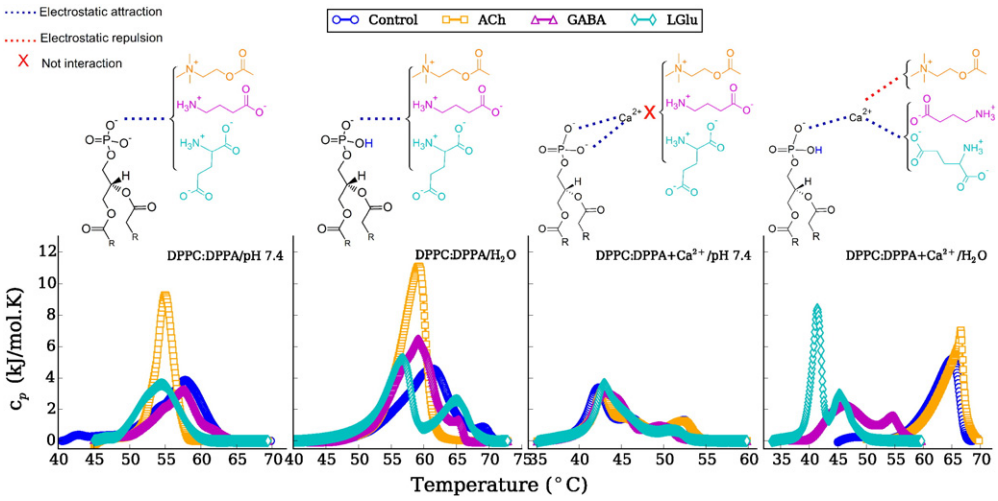

Fig. 6. Changes of calorimetric profiles of the DPPC:DPPA membranes caused by ACh, GABA, and LGlu under different conditions of pH and Ca^{2+} (bottom). The nature of these profiles are electrostatic attraction and repulsion among phosphatidic acid, Ca^{2+}, proton, and neurotransmitters (top).

Acknowledgments

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Appendix A. Supplementary data

Supplementary figures (S1–S4) display total calorimetric enthalpies (area under the curves), for each DSC measurements. This material can be found online. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bbamem.2016.06.017.

References

[1] N. Franks, W. Lieb, Molecular and cellular mechanisms of general anaesthesia, Nature 367 (1994) 607–614.
[2] J.M. Sonner, R.S. Cantor, Molecular mechanisms of drug action: an emerging view, Annu. Rev. Biophys. 42 (2013) 143–167.
[3] A.E. Pereda, Electrical synapses and their functional interactions with chemical synapses, Nat. Rev. Neurosci. 15 (2014) 250–263.
[4] A.L. Hodgkin, A.F. Huxley, A quantitative description of membrane current and its application to conduction and excitation in nerve, J. Physiol. 117 (4) (1952) 500.
[5] T.C. Südhof, R.C. Malenka, Understanding synapses: past, present, and future, Neuron 60 (3) (2008) 469–476.
[6] A. Orłowski, M. Grzybek, A. Bunker, M. Pasenkiewicz-Gierula, I. Vattulainen, P.T. Männistö, T. Róg, Strong preferences of dopamine and L-dopa towards lipid head group: importance of lipid composition and implication for neurotransmitter metabolism, J. Neurochem. 122 (4) (2012) 681–690.
[7] A. Guyton, J. Hall, Organization of the nervous system, basic functions of synapses, and neurotransmitters, Textbook of Medical Physiology, 12th ed.Elsevier Saunders 2011, pp. 543–558.
[8] R. Pérez-Isidoro, F. Sierra-Valdez, J. Ruiz-Suárez, Anesthetic diffusion through lipid membranes depends on the protonation rate, Sci. Rep. 4 (2014).
[9] R. Llinas, M. Sugimori, R. Silver, Microdomains of high calcium concentration in a presynaptic terminal, Science 256 (5057) (1992) 677.
[10] J. Borst, B. Sakmann, Calcium influx and transmitter release in a fast cns synapse, Nature 383 (6599) (1996) 431–434.
[11] E. Neher, Vesicle pools and Ca^{2+} microdomains: new tools for understanding their roles in neurotransmitter release, Neuron 20 (3) (1998) 389–399.
[12] R.C. Lin, R.H. Scheller, Mechanisms of synaptic vesicle exocytosis, Annu. Rev. Cell Dev. Biol. 16 (1) (2000) 19–49.
[13] J. Rizo, C. Rosenmund, Synaptic vesicle fusion, Nat. Struct. Mol. Biol. 15 (7) (2008) 665–674.
[14] E.R. Chapman, How does synaptotagmin trigger neurotransmitter release? Annu. Rev. Biochem. 77 (2008) 615–641.
[15] J. Du, L.R. Reznikov, M.P. Price, X.-m. Zha, Y. Lu, T.O. Moninger, J.A. Wemmie, M.J. Welsh, Protons are a neurotransmitter that regulates synaptic plasticity in the lateral amygdala, Proc. Natl. Acad. Sci. 111 (24) (2014) 8961–8966.
[16] B. Katz, R. Miledi, Further study of the role of calcium in synaptic transmission, J. Physiol. 207 (3) (1970) 789–801.
[17] R.P. Rubin, Historical and biological aspects of calcium action, in: R.P. Rubin, G.B. Weiss, J.W. Putney (Eds.), Calcium in Biological Systems, Plenum Press, New York 1985, pp. 5–11.
[18] R. Rahamimoff, Calcium and transmitter release, in: R. Rahaminoff, B. Katz (Eds.), Calcium, Neuronal Function, and Transmitter Release, Martinus Nijhoff Publishing, Boston, MA 1986, pp. 112–239.
[19] S. Mochida, Presynaptic terminal proteins orchestrate stepwise synaptic vesicle phases, in: S. Mochida (Ed.), Presynaptic Terminals, Springer, Japan 2015, pp. 3–44.
[20] M. Krasowski, N. Harrison, General anaesthetic actions on ligand-gated ion channels, Cell. Mol. Life Sci. 55 (10) (1999) 1278–1303.
[21] A. Scholz, Mechanisms of (local) anaesthetics on voltage-gated sodium and other ion channels, Br. J. Anaesth. 89 (1) (2002) 52–61.
[22] C.J. Weir, The molecular mechanisms of general anaesthesia: dissecting the GABAA receptor, Contin. Educ. Anaesth. Crit. Care Pain 6 (2) (2006) 49–53.
[23] K. Græsbøll, H. Sasse-Middelhoff, T. Heimburg, The thermodynamics of general and local anesthesia, Biophys. J. 106 (10) (2014) 2143–2156.
[24] M. Weinrich, D.L. Worcester, Xenon and other volatile anesthetics change domain structure in model lipid raft membranes, J. Phys. Chem. B 117 (50) (2013) 16141–16147.
[25] G.A. Mashour, S.A. Forman, J.A. Campagna, Mechanisms of general anesthesia: from molecules to mind, Best Pract. Res. Clin. Anaesthesiol. 19 (3) (2005) 349–364.
[26] T. Heimburg, A.D. Jackson, The thermodynamics of general anesthesia, Biophys. J. 92 (9) (2007) 3159–3165.
[27] S.S. Andersen, A.D. Jackson, T. Heimburg, Towards a thermodynamic theory of nerve pulse propagation, Prog. Neurobiol. 88 (2) (2009) 104–113.
[28] R. Appali, U. van Rienen, T. Heimburg, A comparison of the Hodgkin-Huxley model and the soliton theory for the action potential in nerves, Adv. Planar Lipid Bilayers Liposomas 16 (2012) 271–279.
[29] R.S. Cantor, The lateral pressure profile in membranes: a physical mechanism of general anesthesia, Biochemistry 36 (9) (1997) 2339–2344.
[30] R.S. Cantor, Receptor desensitization by neurotransmitters in membranes: are neurotransmitters the endogenous anesthetics? Biochemistry 42 (41) (2003) 11891–11897.
[31] H. Jerabek, G. Pabst, M. Rappolt, T. Stockner, Membrane-mediated effect on ion channels induced by the anesthetic drug ketamine, J. Am. Chem. Soc. 132 (23) (2010) 7990–7997.
[32] S. McLaughlin, The electrostatic properties of membranes, Annu. Rev. Biophys. Biophys. Chem. 18 (1) (1989) 113–136.
[33] A. Verkleij, J. Post, Membrane phospholipid asymmetry and signal transduction, J. Membr. Biol. 178 (1) (2000) 1–10.
[34] W.D. Heo, T. Inoue, W.S. Park, M.L. Kim, B.O. Park, T.J. Wandless, T. Meyer, Pi (3, 4, 5) p3 and pi (4, 5) p2 lipids target proteins with polybasic clusters to the plasma membrane. Science 314 (5804) (2006) 1458–1461.
[35] P.J. Quinn, Plasma membrane phospholipid asymmetry, Phospholipid Metabolism in Apoptosis, Springer 2004, pp. 39–60.
[36] S. McLaughlin, J. Wang, A. Gambhir, D. Murray, Pip2 and proteins: interactions, organization, and information flow, Annu. Rev. Biophys. Biomol. Struct. 31 (1) (2002) 151–175.
[37] S. McLaughlin, D. Murray, Plasma membrane phosphoinositide organization by protein electrostatics, Nature 438 (7068) (2005) 605–611.
[38] T. Yeung, G.E. Gilbert, J. Shi, J. Silvius, A. Kapus, S. Grinstein, Membrane phosphatidylserine regulates surface charge and protein localization, Science 319 (5860) (2008) 210.
[39] M.A. Magalhaes, M. Glogauer, Pivotal advance: phospholipids determine net membrane surface charge resulting in differential localization of active rac1 and rac2, J. Leukoc. Biol. 87 (4) (2010) 545–555.
[40] J.C. Holthuis, A.K. Menon, Lipid landscapes and pipelines in membrane homeostasis, Nature 510 (7503) (2014) 48–57.
[41] D. English, Phosphatidic acid: a lipid messenger involved in intracellular and extracellular signalling, Cell. Signal. 8 (5) (1996) 341–347.
[42] P. Méléard, C. Gerbeaud, P. Bardusco, N. Jeandaine, M. Mitov, L. Fernandez-Puente, Mechanical properties of model membranes studied from shape transformations of giant vesicles, Biochimie 80 (5) (1998) 401–413.
[43] T. Heimburg, Mechanical aspects of membrane thermodynamics. Estimation of the mechanical properties of lipid membranes close to the chain melting transition from calorimetry, Biochim. Biophys. Acta 1415 (1) (1998) 147–162.
[44] D. Murray, A. Arbuzova, G. Hangyás-Mihályné, A. Gambhir, N. Ben-Tal, B. Honig, S. McLaughlin, Electrostatic properties of membranes containing acidic lipids and adsorbed basic peptides: theory and experiment, Biophys. J. 77 (6) (1999) 3176–3188.
[45] T. Heimburg, Membrane structure and the composition of biological membranes, Thermal Biophysics of Membranes, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim 2008, pp. 15–40.
[46] K. Jodko-Piorecka, G. Litwinienko, First experimental evidence of dopamine interactions with negatively charged model biomembranes, ACS Chem. Neurosci. 4 (2013) 1114–1122.
[47] E. Drolle, N. Kučerka, M. Hoopes, Y. Choi, J. Katsaras, M. Karttunen, Z. Leonenko, Effect of melatonin and cholesterol on the structure of dopc and dppc membranes, Biochim. Biophys. Acta Biomembr. 1828 (9) (2013) 2247–2254.
[48] Y. Choi, S.J. Attwood, M.I. Hoopes, E. Drolle, M. Karttunen, Z. Leonenko, Melatonin directly interacts with cholesterol and alleviates cholesterol effects in dipalmitoylphosphatidylcholine monolayers, Soft Matter 10 (1) (2014) 206–213.
[49] P.A. Postila, I. Vattulainen, T. Róg, Selective effect of cell membrane on synaptic neurotransmission, Sci. Rep. 6 (2016) 19345.
[50] D. Papahadjopoulos, W. Vail, C. Newton, S. Nir, K. Jacobson, G. Poste, R. Lazo, Studies on membrane fusion. iii. The role of calcium-induced phase changes, Biochim. Biophys. Acta Biomembr. 465 (3) (1977) 579–598.
[51] H. Binder, O. Zschörnig, The effect of metal cations on the phase behavior and hydration characteristics of phospholipid membranes, Chem. Phys. Lipids 115 (1) (2002) 39–61.
[52] A. Hollander, D. Danino, Cochleate characterization by cryogenic electron microscopy methods: cryo-tem and cryo-sem, Colloids Surf. A Physicochem. Eng. Asp. 483 (2015) 187–192.
[53] R.A. Böckmann, A. Hac, T. Heimburg, H. Grubmüller, Effect of sodium chloride on a lipid bilayer, Biophys. J. 85 (3) (2003) 1647–1655.
[54] R.N. Lewis, D.A. Mannock, R.N. McElhaney, Differential scanning calorimetry in the study of lipid phase transitions in model and biological membranes, in: A.M. Dopico (Ed.), Methods in Membrane Lipids, Humana Press 2007, pp. 171–195.
[55] P.L. Privalov, Calorimetry, Microcalorimetry of Macromolecules: The Physical Basis of Biological Structures, Vol. 16, John Wiley & Soons 2012, pp. 33–68.
[56] A. Scimemi, M. Beato, Determining the neurotransmitter concentration profile at active synapses, Mol. Neurobiol. 40 (3) (2009) 289–306.
[57] M.G. Ganesan, D.L. Schwinke, N. Weiner, Effect of Ca^{2+} on thermotropic properties of saturated phosphatidylcholine liposomes, Biochim. Biophys. Acta Biomembr. 686 (2) (1982) 245–248.
[58] D.R. Slochower, Y.-H. Wang, R.W. Tourdot, R. Radhakrishnan, P.A. Janmey, Counterion-mediated pattern formation in membranes containing anionic lipids, Adv. Colloid Interf. Sci. 208 (2014) 177–188.
[59] Y.-H. Wang, A. Collins, L. Guo, K.B. Smith-Dupont, F. Gai, T. Svitkina, P.A. Janmey, Divalent cation-induced cluster formation by polyphosphoinositides in model membranes, J. Am. Chem. Soc. 134 (7) (2012) 3387–3395.
[60] W. Wang, N.A. Anderson, A. Travesset, D. Vaknin, Regulation of the electric charge in phosphatidic acid domains, J. Phys. Chem. B 116 (24) (2012) 7213–7220.
[61] E.A. Disalvo, L.S. Bakas, Influence of the surface charge distribution and water layers on the permeability properties of lipid bilayers, in: M. Blank (Ed.), Electrical Double Layers in Biology, Plenum Press, New York 1986, pp. 63–76.

[62] C. Wang, F. Ye, G.F. Velardez, G.H. Peters, P. Westh, Affinity of four polar neurotransmitters for lipid bilayer membranes, J. Phys. Chem. B 115 (1) (2010) 196–203.
[63] G.H. Peters, C. Wang, N. Cruys-Bagger, G.F. Velardez, J.J. Madsen, P. Westh, Binding of serotonin to lipid membranes, J. Am. Chem. Soc. 135 (6) (2013) 2164–2171.
[64] D. Papahadjopoulos, K. Jacobson, G. Poste, G. Shepherd, Effects of local anesthetics on membrane properties i. Changes in the fluidity of phospholipid bilayers, Biochim. Biophys. Acta 394 (4) (1975) 504–519.
[65] I. Ueda, C. Tashiro, K. Arakawa, Depression of phase-transition temperature in a model cell membrane by local anesthetics, Anesthesiology 46 (5) (1977) 327–332.
[66] D. Mountcastle, R. Biltonen, M. Halsey, Effect of anesthetics and pressure on the thermotropic behavior of multilamellar dipalmitoylphosphatidylcholine liposomes, Proc. Natl. Acad. Sci. U. S. A. 75 (10) (1978) 4906–4910.
[67] D.B. Goldstein, The effects of drugs on membrane fluidity, Annu. Rev. Pharmacol. Toxicol. 24 (1) (1984) 43–64.
[68] M. Böttner, R. Winter, Influence of the local anesthetic tetracaine on the phase behavior and the thermodynamic properties of phospholipid bilayers, Biophys. J. 65 (5) (1993) 2041–2046.
[69] A.H. Hansen, K.T. Sørensen, R. Mathieu, A. Serer, L. Duelund, H. Khandelia, P.L. Hansen, A.C. Simonsen, Propofol modulates the lipid phase transition and localizes near the headgroup of membranes, Chem. Phys. Lipids 175 (2013) 84–91.
[70] G.H. Peters, M. Werge, M.N. Elf-Lind, J.J. Madsen, G.F. Velardez, P. Westh, Interaction of neurotransmitters with a phospholipid bilayer: a molecular dynamics study, Chem. Phys. Lipids 184 (2014) 7–17.