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In silico investigation of molecular interactions of Volatile Anesthetics: Effects on phospholipid membranes and subcellular targets

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Abstract

The ability of anesthetics to reversibly suppress consciousness must reside in the effects exerted onto specific molecular targets. Interactions between Volatile Anesthetics and the phospholipid membrane as well as intracellular tubulin, were investigated using Computational Molecular Modelling, which showed rapid ligand partitioning inside the membrane and significant effects on the mechanical characteristics thereof, while transient binding locations have been found on the tubulin dimer.

Introduction

Anesthesia, despite being the cornerstone of modern surgery, is to this date a biological puzzle. While scientific efforts still have not managed to pinpoint its exact pharmacological and molecular basis, understanding the effects of Volatile Anesthetics (VAs) on phospholipid membranes and on downstream subcellular molecular targets is a crucial milestone for explaining their complex clinical action. In this context, the interactions between VAs and a model mammalian cell membrane, as well as on human tubulin, the main constituent of microtubules, have been investigated using computational approaches.

Materials and Methods

a) VA-Membrane simulations: model mammalian membranes composed of POPC, POPS, POPE, PSM and Cholesterol at a ratio of 0.265:0.085:0.02:0.29:0.34 (inner leaflet) and 0.1:0.33:0.205:0.025:0.34 (outer leaflet)[1] have been assembled using the CHARMM-GUI webserver and simulated for 1 μs in the NPT ensemble using the CHARMM36[2] forcefield in GRO-MACS 2020.2, at 303.15K and 1 bar. NaCl counterions have been added to reach the physiological salt concentration of 0.15M. Desflurane (DF) and Methoxyflurane (MF) were added in the solvent at 0% (control), 12.5%, 25% and 50% molar ratios with respect to the lipid component. Membrane thickness and area per lipid (APL) were calculated from the simulations. Membrane mechanical stiffness was determined using lipid splay analysis[3] on the MD simulations.

b) VA-Tubulin simulations: models of human tubulin isotypes βVI, βIIa and βIVa from previous work have been simulated in GROMACS for 100ns using the AMBER ff99SB-ILDN force field[4] following the same NPT protocol as described before. Simulations were performed both without and with DF, MF, Ethylene (ET), and Halothane (HT) in the solvent at fixed 10 mM concentration.[5] The main binding clefts were sampled from MD simulations using residence time analysis and the strength of the interaction in different locations has been quantified using the MM/PBSA method.

Results

The interaction of DF and MF with model mammalian membranes takes the form of extended spontaneous membrane partitioning of the ligands, taking place in the first 100ns of MD simulations, consistent with their hydrophobic nature. This behavior has effects on both the APL and membrane thickness. More in detail, the inclusion of both DF and MF causes a reduction in membrane thickness from 46.76 ± 0.29 Å (control) to 45.29 ± 2.10 Å (MF 50%) and 45.67 ± 2.07 Å (DF 50%), as well as an increase in APL from 42.95 ± 1.40 Å^2 (control) to 47.53 ± 2.53 Å^2 (MF 50%) and 47.36 ± 2.34 Å^2 (DF 50%), in a fashion proportional to ligand concentration, as shown in Figure 1 for MF. This is indicative of a progressive contraction of membrane thickness and increased lateral mobility of lipids, as confirmed also by the increasing variability of thickness at higher VA concentrations.

In terms of membrane mechanical bending stiffness, the partitioning of both ligands causes a significant reduction in the monolayer bending modulus from an average of 48.6 ± 0.6 kT (control) to 35.5 ± 0.5 kT (MF 50%) and 34.6 ± 0.5 kT (DF 50%), as highlighted in Figure 2.

The calculated membrane stiffness in control simulations is in good agreement with experimental data, while results in the presence of anesthetics are coherent with previous literature suggesting fluidification and increase of membrane compliance upon ligand partitioning, and consistent with the previously found effects on APL and membrane thickness.

Concerning the analysis of the subcellular interaction with tubulin, anesthetics showed distinct interaction patterns with human tubulin dimers, except for ET, which represents the negative control since it is the least potent anesthetic, showing no significant binding. Results confirm the existence of preferential transient binding pockets on the tubulin dimer, and the analysis of average residence time and estimated binding affinity confirmed the lack of interaction with ET: while DF, HT and MT showed comparable affinities between -7 and -14 kcal/mol, predicted affinities for ET were consistently below 4 kcal/mol, indicative of no significant interaction.

Discussion and Conclusions

The results suggest that in computational models of mammalian cell membranes, anesthetics rapidly partition in the lipid bilayer, and their presence can significantly alter the overall lipid behavior and membrane mechanics, in the form of a reduction of bending stiffness of more than 10 kT. This modification might have profound consequences not only on membrane
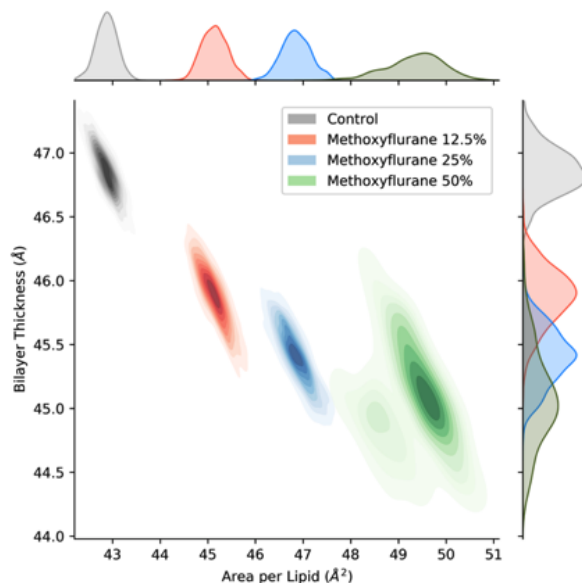
Figure 1. Effect of MF on membrane thickness (y axis) and APL (x axis) at different concentrations.

Figure 2. Effect of MF and DF on membrane stiffness as a function of anesthetic concentration.

behavior itself, but also on the mechanics of channel proteins, currently regarded as the main molecular targets of anesthetics. Inside the cell, on the cytoskeleton, the lack of a unique and stable predicted binding site for volatile anesthetics on tubulin suggests that binding does not follow a lock-and-key paradigm, but can occur repeatedly inside different, energetically favorable clefts, with possible consequences including cross-interactions with MT-targeting chemotherapeutic agents or the exacerbation of side effects of anesthesia, especially in the context of MT-altering neurodegenerative diseases.

A better understanding of the molecular interactions of anesthetics, both in terms of their main mechanism of action and possible off-target interactions, could pave the way for the design of novel anesthetic molecules with improved pharmacology and reduced side effects, through preliminary *in silico* investigations, reducing unneeded *in vitro* and *in vivo* trials.

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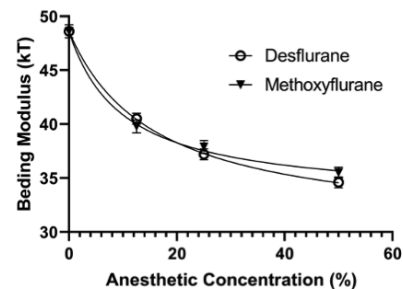1. Shahane G, Ding W, Palaiokostas M, et al. Physical properties of model biological lipid bilayers: insights from all-atom molecular dynamics simulations. J Mol Model 2019;25:76.
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