Sevoflurane and Isoflurane effects on lipid membrane fluidity at clinical concentrations

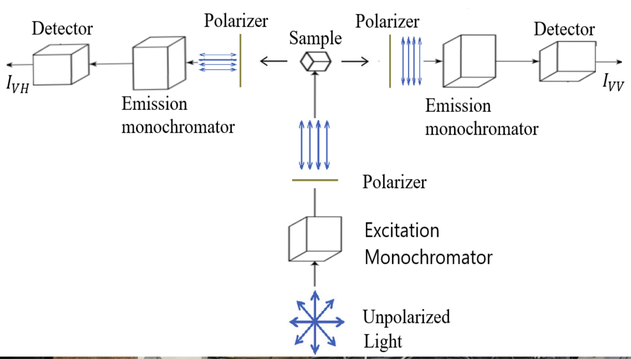
Muhammad Bilal Siddique and Dr. Juyang Huang
Department of Physics and Astronomy, Texas Tech University

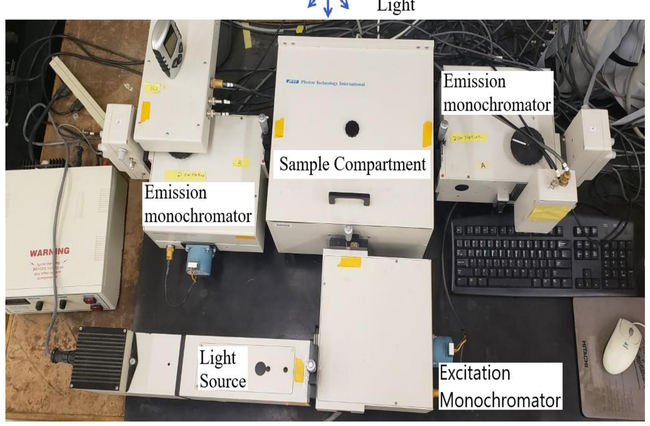
Introduction

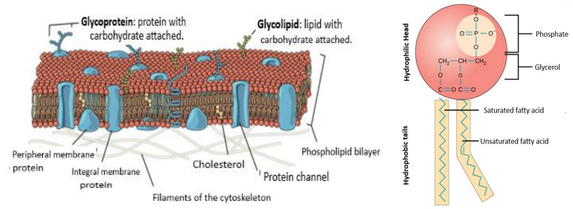
In this study, the effects of anesthetic drug sevoflurane on membrane fluidity are compared with isoflurane. To get complete picture, three representative membrane systems (pure DPPC, POPC/Chol and a 5-lipids mixture that mimics brain endothelial cell membrane) and red blood cells were chosen. Lipid membrane systems were labeled with dipyrene-PC fluorescent probe, whose excimer/monomer (E/M) fluorescence peak ratio showed an immediate increase after adding the drugs, indicating a sharp increase of membrane fluidity. We studied clinical concentrations of 0.5mM sevoflurane and 1mM isoflurane. The fluidity increases at these concentrations on DPPC lipid bilayer are comparable, and both drugs are quite effective to loosen up the highly ordered lipid domains of saturated lipids. The supra-clinical concentrations of these drugs, 2mM sevoflurane and 5mM isoflurane, have also been examined. The E/M ratio increases for POPC/Chol and 5-lipids mixture were similar, but the magnitude of increases were reduced to almost half of DPPC. Furthermore, washed human red blood cells were labeled with TMA-DPH fluorescent probe and fluorescence anisotropy measurements were carried out. At clinical concentrations, the decreases of anisotropy were comparable for these two drugs, and the effects are more than that of 174mM ethanol, which is ten times the legal alcohol limit level in human blood. All these findings depict that sevoflurane and isoflurane at clinical concentrations have similar effects on a wide range of membrane systems, and both significantly and rapidly increase membrane fluidity.

Materials and Methods

- We fabricated three types of model membranes; pure DPPC (gel-phase at 37°C), BSM/POPC/POPS/POPE/Chol=14.5/31/7.4/24.6/22.5 (that mimics brain Endothelial cell Membranes) and POPC/Chol=90/10 (L_d phase) .
- Each membrane sample was labeled with 0.2% Dipyrene-PC fluorescent probe.
- We washed human red blood cells (RBC) and labeled with TMA-DPH fluorescent probe.
- Two anesthetic drugs; Sevoflurane and Isoflurane were tested on model membranes and on RBC.
- A PTI-spectrophotometer was used to carry out E/M ratio experiment (without polarizers) and TMA-DPH fluorescence anisotropy (with polarizers).

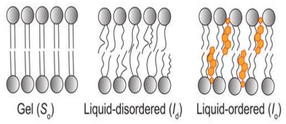




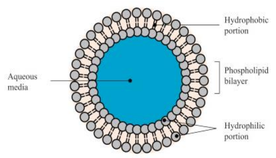


Cell Membrane

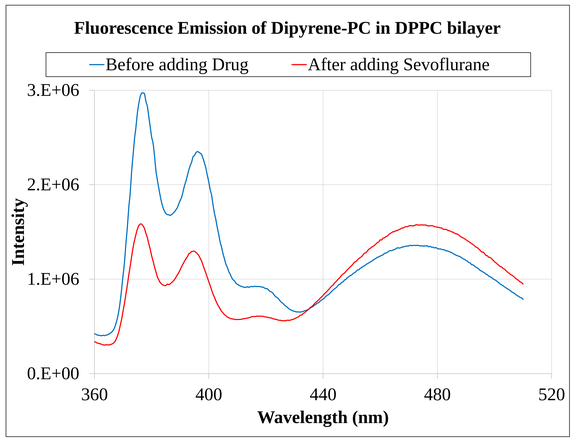
Phospholipid

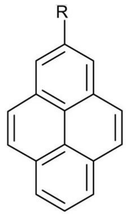


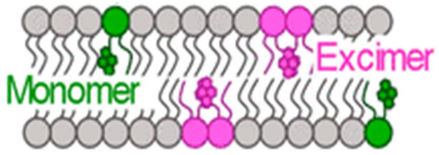
Different states of Lipids



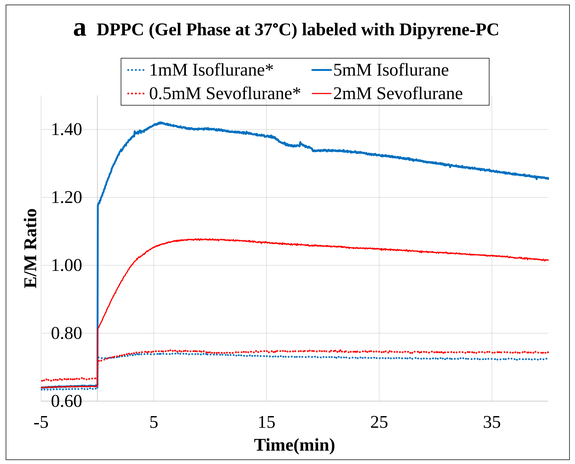
Liposome: A model Membrane

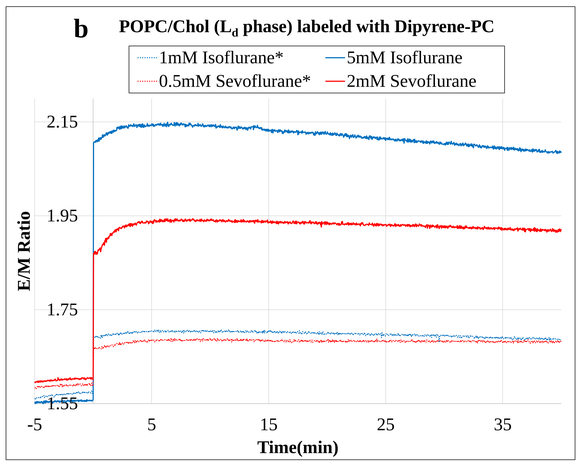


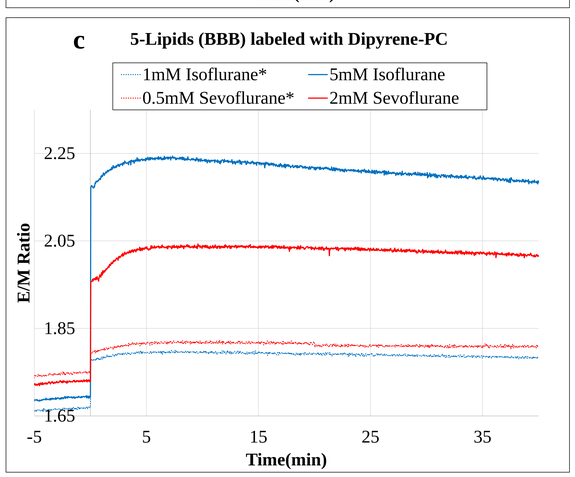


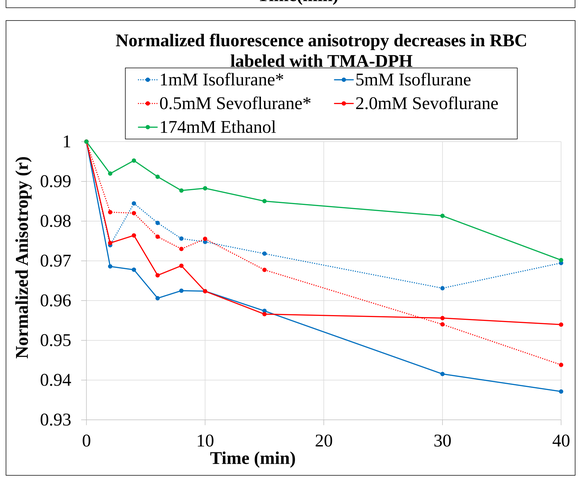


Pyrene labeled Lipids









Results and Discussion

- The emission spectra of Dipyrene-PC in DPPC lipid bilayer before and after adding Sevoflurane is shown in figure.
- Monomer emission and peaks are acquired at 376nm and 398nm while excimer is marked with a broad peak centered at 470nm.
- The E/M ratio of Dipyrene-PC, which is defined as the ratio of excimer peak intensity at 470nm to monomer peak intensity at 376nm, is sensitive to membrane fluidity.
- When a membrane becomes more fluid, it leads to the formation of more excimers and E/M ratio is increased. It can also be seen in the figure that the E/M ratio is instantly increased after adding drugs.

Dipyrene E/M ratio Experiments

Cell Membranes	Drug Concentrations		Average of 5 min before adding Drug	Average of 20 min after adding Drug	% change
DPPC	Sevoflurane	0.50mM*	0.664±0.028	0.743±0.023	11.9%
		2.0mM	0.642±0.001	1.041±0.028	62%
	Isoflurane	1mM*	0.636±0.009	0.734±0.005	15.4%
		5mM	0.643±0.004	1.372±0.041	113.4%
POPC/Chol	Sevoflurane	0.50mM*	1.588±0.008	1.683±0.006	6.0%
		2.0mM	1.601±0.011	1.933±0.041	20.7%
	Isoflurane	1mM*	1.569±0.010	1.702±0.010	8.5%
		5mM	1.555±0.019	2.135±0.056	37.3%
5-Lipids	Sevoflurane	0.50mM*	1.746±0.022	1.815±0.024	3.9%
		2.0mM	1.727±0.006	2.028±0.021	17.4%
	Isoflurane	1mM*	1.666±0.053	1.793±0.043	7.6%
		5mM	1.690±0.010	2.227±0.016	31.8%

- The fluorescence anisotropy is defined as:

$$r = \frac{I_{VV} - gI_{VH}}{I_{VV} + 2gI_{VH}} \; with \; g = \frac{I_{HV}}{I_{HH}}$$

Where I_{VV}, I_{VH} are the emission intensities of vertical and horizontal polarized light

TMA-DPH Anisotropy of RBC

Drug Concentration	Before adding Drug	Average of 20 min after adding Drug	Percentage Change
1mM Isoflurane*	0.285177±0.0011	0.278532±0.0018	-2.33%
5mM Isoflurane	0.285858±0.0040	0.275341±0.0052	-3.68%
0.5mM Sevoflurane*	0.287785±0.0040	0.280907±0.0035	-2.39%
2.0mM Sevoflurane	0.284942±0.0083	0.275681±0.0089	-3.20%
174mM Ethanol	0.302055±0.0130	0.299003±0.0123	-1.01%

*clinical concentrations of anesthetic drugs

Conclusion

In this study, the effects of anesthetic drugs on lipid membrane fluidity was measured using Dipyrene-PC E/M ratio experiments and TMA-DPH fluorescence anisotropy. The key findings are: (1) Sevoflurane and Isoflurane increase the membrane fluidity at their clinical concentrations. (2) Their clinical effects are comparable and show maximum change in simple bilayer of DPPC. They are quite effective to loosen up the highly ordered saturated lipids. (3) It is likely that they are also effective to increase the permeability of blood brain barriers. (4) Even at the low clinical concentrations of the drugs, the membrane fluidity increases in human RBC are larger than that produced by 174mM ethanol, which is 10-times the legal limit of alcohol in human blood.

References:

JN.P. Franks, W.R. Lieb, Mechanisms of general anesthesia, Environ. Health Perspect. 87 (1990) 199–205.

S. Mukherjee, W. Xu, F.F. Hsu, J. Patel, J. Huang, K. Zhang, Sterol methyltransferase is required for optimal mitochondrial function and virulence in Leishmania major, Mol. Microbiol. 111 (2019) 65–81.

J.T. Buboltz, A more efficient device for preparing model-membrane liposomes by the rapid solvent exchange method, Rev. Sci. Instrum. 80 (2009).