The effect and functioning of general alcohol anesthetics

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To determine if anesthetics function by influencing liquid ordered – liquid disordered membrane lipids

To determine how anesthetics affect the degranulation signaling pathway
How do anesthetics work?

- Do they directly affect proteins?
- Do they indirectly affect proteins by changing the physical properties of surrounding lipids?
- Even within the field of lipids there is debate

Membrane protein theory of the anesthetic effect

Lipid theory of the anesthetic effect

Alcohol anesthetics act in a non-specific manner by integrating into the membrane to cause changes to the conformational equilibrium of lipids and proteins in the membrane.

To test this, we will be seeing if there is a correlation between the anesthetic effect on phase transitioning and a functional signal pathway.
How to test hypothesis

- The model system
  - Using blebs of rat basophilic leukemia (RBL) mast cells (white blood cells responsible for immune responses)
  - Observing phase transition temperature of blebs treated with several concentrations of different alcohols

- The signaling pathway
  - Using the degranulation immune response signaling pathway, as a system that has nothing to do with nerve responses or ion channels
  - Observing how alcohols affect certain stages in this pathway

- Connection between model system and degranulation
  - If alcohols have a similar effect on both experiments, correlations can be made providing evidence that general anesthetics have an indirect effect on proteins via lipid membranes.
Alcohols as liquid anesthetics

- The concentrations that are used in the following experiments are based on a paper published by Michael J. Pringle et. al.
- Determined concentration (AC50) for each alcohol at which 50% of tadpoles lost the righting reflex (the response allowing animals to properly orient their bodies)
- For our purposes, this concentration is termed “1X” and each multiple of this value is “2X”, “5X” etc.
By treating RBL cells with formaldehyde and Dithiothreitol (DTT), the cells release blebs that can be used as more simple model membranes.

Membrane is labeled with Dil-C12, which prefers to partition into liquid disordered regions.

As temperature is decreases, blebs phase separate into distinct light phase (liquid disordered) and dark phase (liquid ordered) regions.

Matcha, Minimal model of plasma membrane heterogeneity requires coupling cortical actin to criticality., 2011, Biophys. J.
Analysis of phase transitioning

Although each bleb has a clearly defined phase transition temperature, the population of blebs does not.

By counting the number of phase separated blebs for each image, a sigmoidal curve representing phase transitioning is produced.

The temperature at which 50% of the blebs are phase separated is known as the transition temperature.
The blebs are treated with various multiples of a pre-determined concentration (AC50) for each given alcohol (ethanol, propanol, octanol, decanol), which lowers the phase transition temperature.

Control
0.5X ethanol
1X ethanol
2X ethanol
5X ethanol
Points represent the difference in critical temperature between the experimental (added alcohol) and the control (no anesthetic)

The decrease in transition temperature as concentration increases indicated the interference in membrane composition when alcohols are added

When dividing by the AC50, all alcohols tested had a very similar effect

DMSO does not have a documented anesthetic effect so it is being used as a control

Our data shows that DMSO has no effect, indicating that the effect seen by the alcohols is significant
Degranulation is an allergic response signaling pathway which is IgE receptor-mediated.

Used as a cell functionality assay.

How we are using this signaling pathway

- Well documented that anesthetics have an effect on ion channels
- Demonstrating a clear effect on this unrelated pathway may suggest that it is a physical property of anesthetics that causes their effect rather than a more specific property.
Absorbance assay that detects a specific enzyme (β-hexosaminidase) released in the granules

Observing a late stage of the signaling pathway

(Mark, Crystallographic Evidence for Substrate-assisted Catalysis in a Bacterial β-Hexosaminidase, 2001, J. Bio. Chem.)
For each given concentration of each anesthetic, the following set-up was used:
## Degranulation data

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**ETHANOL**

**PROPANOL**

**OCTANOL**

**DECANOL**
Degranulation Data

However, there are several outliers, particularly for decanol, primarily due to the fact that many alcohols are quite volatile.

- Ethanol
- Propanol
- Octanol
- Decanol

Most of the points follow a steadily decreasing absorbance amount as concentration increases.
The exocytosis of granules is affected by anesthetic alcohols in a comparable way to how transition temperature is affected

Provides more evidence that anesthetic may be effecting cell functioning in an indirect manner through the lipid component of membranes
Future direction...

- We can probe earlier parts of pathway
  - Calcium released from ER
  - Activation of receptors in the membrane
Using transition temperature of blebs, we showed that alcohols lowered the transition temperatures by similar amounts for each given AC50 value.

Degranulation decreased in a similar manner for increasing concentrations of alcohols.

Future experiments will provide further evidence for the functioning of general anesthetics.

Making the correlation between degranulation and phase transition provides more evidence that the liquid-liquid phase transitions is key to the functioning of anesthetics and that general anesthetics act indirectly on proteins through this method.
Questions?