BBA - Biomembranes xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Biomembranes

journal homepage: www.elsevier.com/locate/bbamem

# The free energy of biomembrane and nerve excitation and the role of anesthetics☆

Tian Wang, Tea Mužić, Andrew D. Jackson, Thomas Heimburg*

*Membrane Biophysics Group, Niels Bohr Institute, University of Copenhagen, Denmark*

ARTICLE INFO

*Keywords:*
Lidocaine
Calorimetry
Stimulation-response profiles
Heat capacity
Phase transition

ABSTRACT

In the electromechanical theory of nerve stimulation, the nerve impulse consists of a traveling region of solid membrane in a liquid environment. Therefore, the free energy necessary to stimulate a pulse is directly related to the free energy difference necessary to induce a phase transition in the nerve membrane. It is a function of temperature and pressure, and it is sensitively dependent on the presence of anesthetics which lower melting transitions. We investigate the free energy difference of solid and liquid membrane phases under the influence of anesthetics. We calculate stimulus-response curves of electromechanical pulses and compare them to measured stimulus-response profiles in lobster and earthworm axons. We also compare them to stimulus-response experiments on human median nerve and frog sciatic nerve published in the literature.

## 1. Introductions

The nervous impulse is most commonly recognized as a phenomenon related to current and voltage changes [1]. It is less known that the nerve pulse also has a mechanical [2-5] and a thermal component [6-10]. Based on the latter findings, it has been proposed that the nerve pulse possesses features of a solitary electromechanical pulse [11-15]. The theory is based on the nonlinear (electro-) mechanical properties caused by the melting transition of biological membranes from an ordered gel to a disordered fluid state [16,17]. In particular, membranes display a larger lateral compressibility in a transition [18]. In biological membranes, this transition typically occurs in a temperature regime 10–15° below physiological temperature [16]. Since this phase transition depends on temperature, pressure, voltage and the chemical potential of drugs such as anesthetics (actually, the chemical potentials of all membrane components including proteins and cholesterol), the free energy necessary to induce it depends on these intensive variables. Interestingly, it is well-known that changes in these variables either excite nerves or inhibit them. Determining the free energy required to excite a membrane transition is the topic of this paper.

Gel membranes display a larger volume and area density than fluid membranes. For this reason, a transition in a membrane from fluid to gel can be induced by changes in hydrostatic [19-21] or lateral pressure. The latter has been extensively studied on monolayers [22-24], where a liquid expanded to liquid condensed phase transition is found with increasing lateral pressure. Close to transitions, the response of the membrane to changes in lateral pressure is nonlinear. This gives rise to the emergence of density pulses in membranes which resemble solitons or solitary waves [11,12,15]. Such pulses have been found experimentally in monolayers close to transitions [25-28], and electromechanical behavior has also been observed in living nerves [2-5,10].

The features of these solitary pulses depend largely on the properties of the melting transition in the membranes and in particular on the distance of the transition from physiological temperature. Any variable that influences the position of the transition relative to physiological conditions potentially affects the excitability of the membrane [29,30]. Besides changes in ambient temperature, this applies most notably to changes in hydrostatic or lateral pressure, pH, calcium and other salt concentrations [31,32] and the addition of small drugs such as anesthetics [29,33], which are all known to influence the transition temperature.

The example of general anesthetics is of particular interest. It has long been known that general anesthetics obey the Meyer-Overton correlation [33-37]. This correlation expresses the fact that the potency of an anesthetic is proportional to its solubility in the lipid membrane of cells. It can be expressed as $P \cdot [ED_{50}] = \text{const.}$, where $P$ is the partition coefficient of the anesthetic in the membrane, and $[ED_{50}]$ is the free anesthetic concentration for which 50% of all individuals are anesthetized [29]. The critical dose of anesthetics in the lipid membrane is thus the same for all general anesthetics. This has important consequences, e.g., the known additivity of the effect of different anesthetics. The Meyer-Overton correlation is an empirical finding that does not in itself

☆ This article is part of a Special Issue entitled: Emergence of Complex Behavior in Biomembranes edited by Marjorie Longo.

* Corresponding author.
*E-mail address:* theimbu@nbi.ku.dk (T. Heimburg).

https://doi.org/10.1016/j.bbamem.2018.04.003
Received 30 January 2018; Received in revised form 26 March 2018; Accepted 11 April 2018

represent an explanation. The fact that anesthetics dissolve in membranes does not imply a mechanism. However, it has been shown that the Meyer-Overton correlation acquires a physical meaning if one takes into account the fact that general anesthetics dissolve only in fluid membranes and not in gel membranes [29,33]. It has been shown that general and local anesthetics induce a melting point depression in the membrane that is described by [29,30]

$$\Delta T_m = -\frac{RT_m^2}{\Delta H} x_A \tag{1}$$

where $T_m$ is the melting temperature, $\Delta H$ is the latent heat of the phase transition, and $x_A$ is the molar fraction of anesthetics in the lipid membrane. At critical dose, $x_A$ and $\Delta T_m$ are always the same ($\approx$ 2.7 mol % and $-0.6^\circ$, respectively [29]).

The lowering of the melting temperature by anesthetics can be compensated by the application of hydrostatic pressure [29,38-41]. For tadpoles, the critical pressure at which anesthetized tadpoles recover is about 50 bars. It can be calculated from the known pressure dependence of the melting temperature of lipid membranes [29], which suggests that the melting transition is somehow coupled to the function of nerves.

The temperature, pressure, lateral pressure and anesthetic dependence of the chemical potential difference between fluid and gel membrane, $\mu_{gf}(T,\Delta p,\Delta\Pi,x_A)$, is given by [29]:

$$\Delta\mu_{gf} \approx \Delta H\left(\frac{T_m - T}{T_m} + \gamma_V \Delta p \frac{T}{T_m} + \gamma_A \Delta\Pi \frac{T}{T_m} - \frac{RT}{\Delta H} x_A + \ldots\right) \tag{2}$$

where $\Delta p$ is a change in hydrostatic pressure, $\Delta\Pi$ is a change in lateral pressure in the membrane plane, and $\gamma_V = 7.8 \cdot 10^{-10}$ m$^2$/J (for DPPC) and $\gamma_A = 0.89$ m/J are coefficients related to volume and area expansion of the lipids in the transition. The above equation contains a term for each intensive variable that is controlled during the experiment. For this reason, the free energy difference also depends on the transmembrane voltage [42,43], even though the exact functional form of the term remains to be explored. Any membrane component such as proteins, cholesterol, the proton concentration (pH), and so forth, adds a term as long as it is controlled from the outside prior to the experiment. In the present paper we focus on changes of lateral pressure in pulses and the role of anesthetics that display an especially large influence on the free energy differences.

In the soliton theory for nerve pulse propagation, the pulse consists of a segment of solid phase traveling in the otherwise liquid membrane. The free energy for exciting a pulse is therefore related to the chemical potential difference for the conversion of fluid membrane to gel membrane.

The free energy difference between a fluid and a gel membrane is given by

$$\Delta G_{gf} = f \cdot \Delta\mu_{gf}, \tag{3}$$

where f is the fraction of fluid lipid. The free energy necessary to convert a membrane at $T,p,\Pi,x_A$ from fluid to gel is therefore given by

$$\Delta G_{ex} = -\Delta G_{gf}. \tag{4}$$

This is the free energy required to excite a soliton. It depends on the intensive variables, e.g., voltage, lateral pressure, temperature or the chemical potentials of anesthetics. The dependence on anesthetic concentration will be explored below.

## 2. Materials and methods

### 2.1. Chemicals

Lidocaine (98%), NaCl, KCl, $CaCl_2$, $MgCl_2$, Tris, and glucose were purchased from Sigma-Aldrich. All water used was Milli Q water (18.1 MΩ) prepared by a Direct-Q® 3 UV water purification system (Merck).

### 2.2. Porcine spine membranes

Porcine spine was acquired from the local butcher. It was kept in a refrigerator and was never frozen. The spinal cord was homogenized using a rotor-stator 125 Watt Lab Homogenizer with 7 mm Probe (Tissue Master, Omni International Inc., Kennesaw, GA) at 33,000 RPM in 30-s intervals for about 30 min. The homogenized tissue was diluted with a 150 mM NaCl, 10 mM $Na_2HP0_4$ and 1.8 mM $KH_2P0_4$ buffer at pH 7.4. The homogenized sample was filtrated through stainless steel 100 mesh with 140 µm opening size (Ted Pella, Inc., Redding, CA) in order to remove large fibers. Samples were centrifuged at 20,800 RCF for 10 min in an Eppendorf desk centrifuge. After centrifugation one finds a pellet of membranes. The supernatant was discarded in order to remove soluble proteins and other soluble components. The removed supernatant was replaced with buffer, vortexed and centrifuged again. This procedure was repeated five times. The pellet was assumed to contain the clean spinal cord membranes, which is evident from the heat capacity profile containing a pronounced lipid melting peak. No lipids were added to the sample, and no proteins removed. The membranes should display the same composition as in the native situation. More details can be found in [44].

### 2.3. Earthworm preparations

Earthworms (*Lumbricus terrestris*) were obtained from a local supplier. The procedure for extracting the nerve is described in detail in [45]. We used an earthworm saline solution adapted from [46] consisting of 75 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Tris, and 23 mM glucose, adjusted to pH 7.4.

### 2.4. Lobster preparations

Lobsters *Homarus americanus* were acquired from a local supplier. Nerves were extracted following a procedure described in [45]. We used a lobster saline solution adapted from [47] (462 mM NaCl, 16 mM KCl, 26 mM $CaCl_2$, 8 mM $MgCl_2$, 10 mM TRIS and 11 mM Glucose, adjusted to pH 7.4.

### 2.5. Nerve recordings

Extracellular recordings of the action potential were performed using a Powerlab 26T (ADInstruments, Australia), an integrated data acquisition system with built-in function generator. Details are given in [45].

### 2.6. Calorimetry

Calorimetric scans were performed on a MicroCal VP-DSC (Northampton, MA) differential scanning calorimeter. Heating rates were kept at 20° C/h, filtering period was 5 s and feedback was set to none. Prior to the experiment, samples were degassed for a few minutes under light vacuum.

## 3. Theory

### 3.1. Electromechanical solitons

The fluctuation-dissipation theorem implies that fluctuations in enthalpy are proportional to the heat capacity, while fluctuations in volume and area are proportional to their respective compressibilities [18]. Furthermore, volume, area and enthalpy are proportional functions of temperature [18,48]. This convenient fact allows us to calculate the compressibility of membranes from the excess heat capacity. One finds that the compressibility is a proportional function of