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POTENTIAL DEPENDENT RIGIDITY CHANGES IN LIPID MEMBRANE VESICLES

Peter I. Lelkes

Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel

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SUMMARY : Steady-state fluorescence depolarization measurements of the hydrophobic probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), embedded into lipid membrane vesicles, reveal an increase in the membrane rigidity in the presence of transmembrane potential gradients. The effect depends on the lipid head-group structure and its charge. Additionally, the curvature of the vesicle membrane leads to an asymmetry in the observed phenomena. In consistence with several theories and experiments, it is concluded that besides a possible electrostriction of the membrane the electric field causes alterations in the orientation of the surface dipoles of the lipid molecules. The biological significance of these observations is briefly discussed.

The dependence of membrane structure on the transmembrane electrical potential is of general interest in biology, especially in the field of electrophysiology. It has been suggested that the molecular mechanism of nervous conduction is partially based on conformational changes in the nerve membrane, which are caused by action potentials involving transient changes in the electric field over the membrane in the order of 10^5-10^6 V/cm (1,2).

Several hypotheses have been presented and partially verified experimentally, which indicate that the membrane structure might undergo appreciable modifications by an imposed potential gradient (3-8). Early attempts to monitor these structural changes via external optical probes were deserted, mainly because these probes seemed to be sensitive to the potential gradient across the membrane rather than to structural modifications of the membrane itself (1,9). However, while following phase transitions in lipid vesicles with the aid of one of these "potential" probes (10) we found that the mode of action of the dye Merocyanine 540, a polar dye located in the head-group region of the phospholipids, is remarkably similar while used both as a potential and as a structural probe. We thus concluded

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that, besides its direct interaction with the electric field (9,11), this dye should also sense and report on structural changes involving the membrane fluidity, that are caused by transmembrane potentials.

In order to experimentally verify our assumption of potential-induced fluidity changes and to rule out possible misinterpretations due to the alignment of a polar dye in the electric field, we measured the electric field-dependence of the steady-state fluorescence depolarization of the hydrophobic probe DPH[1]. Transmembrane potentials due to Na^+/K^+ gradients were created, using NaCl and KCl and valinomycin as K^+-selective ionophore. In similar experiments carried out in our department, Na^+/K^+ gradients are formed with impermeable anions (Pasternak *et al.*, to be published).

MATERIALS AND METHODS

Phosphatidylcholine (PC) and phosphatidylserine (PS) were purchased from Lipid Products, Nutfield, England. H_2O was bidistilled, all inorganic reagents used were of p.A. grade (Merck). The preparation of single-walled, sonicated vesicles was described earlier and followed standard procedures (10). Labelling of the lipid vesicles with DPH (Fluka, puriss) followed standard procedures (12).

All experiments were performed in a 10^{-3}M phosphate buffer (pH = 7.0±0.1), containing a total of 400 mOsmole of KCl and NaCl. While replacing KCl by NaCl we varied $[K^+]$ inside and outside the vesicles between 0 and 200 mM. In order to establish the desired ion gradients the vesicles were passed through a Sephadex G-25 column, using eluents with different $[K^+]^o$. In another set of experiments, which yielded essentially the same results, the internal potassium concentration $[K^+]^i$ in the vesicles was varied between 10^{-6} and 10^{-3}M and aliquots of the vesicles were transferred directly from the electrolytes, in which they were prepared, into the desired media. In this range of salt concentration the effect of osmolarity upon membrane fluidity is negligible (14).

An electric field across the membrane was generated by adding Valinomycin (Sigma) at a concentration of 10^{-7}M (molar ratio lipid:valinomycin ≥1000:1). The membrane potential ψ was calculated according to the Nernst equation as $\psi = 58 \log([K^+]^o/[K^+]^i)$.

The mathematical formalism relating the steady-state fluorescence depolarization of DPH to membrane fluidity has recently been reviewed (12). The microviscosity parameter (equation 14 in 12), termed rigidity, R, in the present communication, is related to the structure of both the dye

1. Due to its rodlike structure DPH appears to be a more suitable indicator of membrane fluidity than flat, disc-like probes, like e.g. pyrene (12), with which Georgescould *et al.* recently attempted to monitor fluidity changes in nerve axons during an action potential (13).

and the membrane, in which it is embedded. By using this term, one can avoid some disputed approximations (15,16) connected with the "apparent microviscosity". In consistence with the notation of optical changes due to transmembrane potentials (9) we present our data as relative changes in rigidity $\Delta R/R_0$ with $\Delta R = R(\psi) - R_0$. $R(\psi)$ is the rigidity in presence of an electric field, measured 5 min after addition of the ionophore and R_0 is the rigidity in the absence of the transmembrane potential gradient.

The experiments were performed at room temperature ($23\pm1^{o}C$) on a Hubweiz Fluorescence Fluorimeter (Flupol IV, Hubrecht, Labs., Utrecht, Holland) equipped with all the necessary optics for working with DPH as a fluidity probe (17). The data were recorded as the ratio of horizontally/vertically polarized fluorescence $I_{\shortparallel}/I_{\perp}$ from which the rigidity was calculated (12). Control experiments were performed according to (18) to rule out possible artifacts.

RESULTS AND DISCUSSION

Figure 1 demonstrates the dependence of the relative change in membrane rigidity ($\Delta R/R_0$) on the transmembrane potential ψ for PC and PS vesicles. A positive potential is assigned for $[K^+]^o/[K^+]^i>1$; the different symbols represent data from several independent experiments. With increasing membrane potential the relative rigidity changes increase nonlinearly both for negative and positive membrane potentials. Additionally, this behaviour

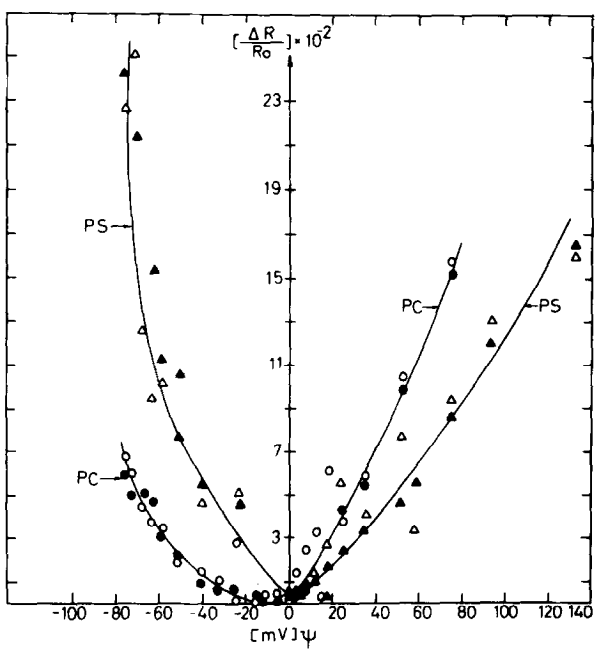

Figure 1 : The relative change in membrane rigidity $\Delta R/R_0$ in dependence of the transmembrane potential ψ, measured in phosphatidylcholine (PC) and phosphatidylserine (PS) vesicles. The different symbols represent independent experiments. For details, see text.

is asymmetric with respect to zero membrane potential for a given lipid species and depends also on the electrical charge in the polar-head group region of the phospholipids. If the observed effects simply originated in an alignment of the DPH-molecules in the electric field and a subsequent decrease in their rotational mobilities, and we should expect a linear dependence of the optical signal on the square of the electric field (19). Figure 2 demonstrates that this is not necessarily the case. Besides numerical differences between PS and PC, there are close similarities in shape of the ψ^2-dependence in both lipids and for both positive and negative potentials. The initial linearity in the $\Delta R/R_o(\psi^2)$ curves for small potential differences indicates a certain direct interaction between the electric field and the dye molecules. However, for appreciable potential gradients across the lipid membranes, the significant and polarity-specific deviations from linearity in Figure 2 suggest that in addition to a possible interaction between the electric field and DPH via induced dipole moments, other parameters, directly connected to the membrane structure, have to be considered. The dependence on the head-group structure leads to the conclusion that in lipid-bilayers the transmembrane potential interacts strongly with the electrically polarizable parts of the lipid molecules, especially the surface dipoles. The asymmetry in the $\Delta R/R_o(\psi)$ curves might originate in the structural asymmetry of vesicular bilayers due to the stronger curvature of the interior monolayer (20).

The role of surface dipoles in the interaction of biological membranes with electric fields has been pointed out in several theoretical calculations and it was postulated that field-induced changes in the dipole-orientation might be part of the molecular mechanism underlying the nervous excitation (3,4). Experimental evidence for these assumptions is found i.a. in changes in the infrared spectrum of nerve axons during excitation (21,22), suggesting that concomitant with the action potential conformational changes occur in the phospholipid constituents of the nerve membranes and especially

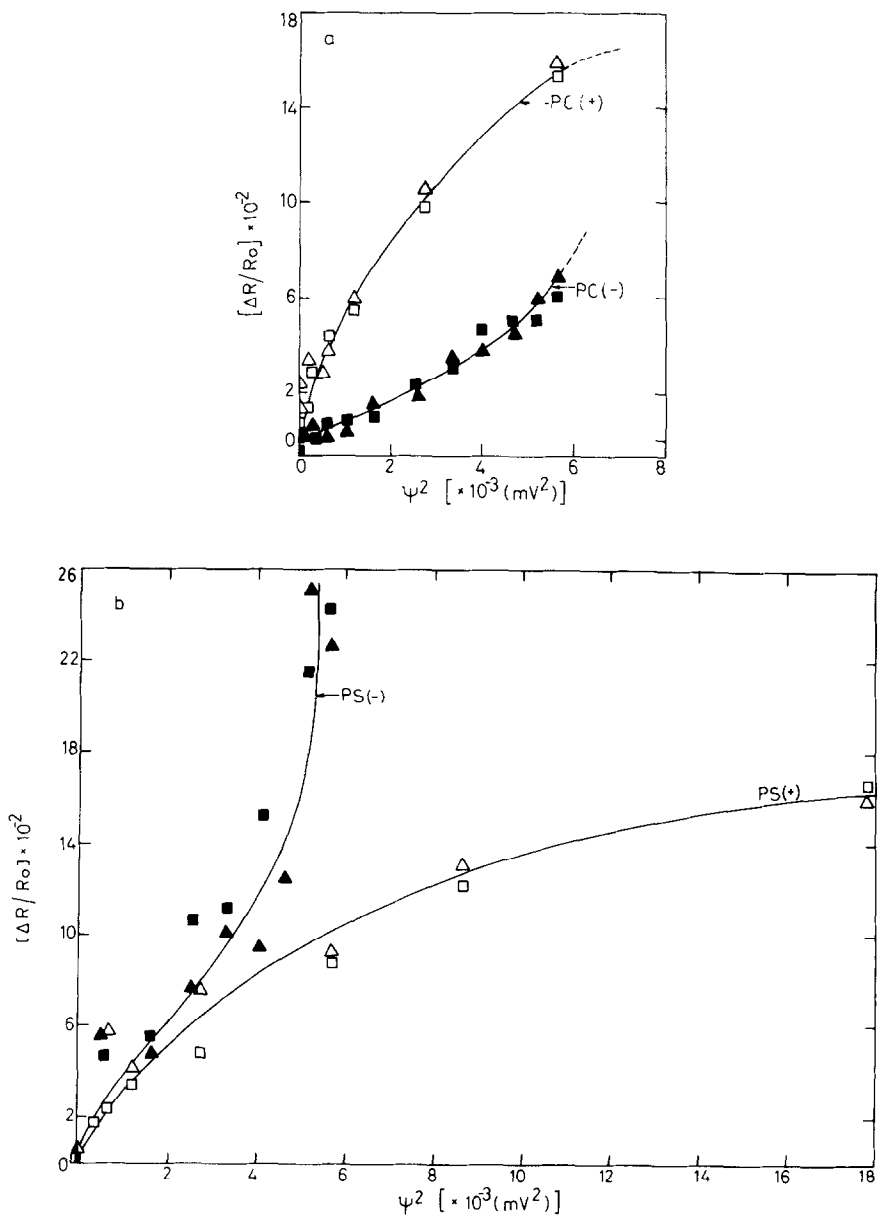

Figure 2 : $\Delta R/R_o$ values from Figure 1, plotted against the square of the membrane potential (ψ^2), for positive (+) and negative (-) directions of the electric field vector. Details in the text. (a) Phosphatidylcholine; (b) Phosphatidylserine vesicles.

within their head-groups. In similar experiments, Cohen and his coworkers (23,24) investigated changes in intrinsic optical parameters (light scattering and birefringence), that accompany the action potential in squid giant axons, a dependence of the optical signals on the square

of the membrane potential. The extreme of these parabolic curves are situated at potentials very close to zero membrane potential. These results, that are not hampered by membrane asymmetries due to the different curvatures of the outer and the inner lipid monolayers, indicate the occurrence of structural alterations in the presence of an electric field. Cohen *et al.* discuss their results in terms of a Kerr-effect like modification of the membrane structure which could involve both an electrostriction of the membranes and/or a reorientation of membrane-bound dipoles.

In this communication we present experimental evidence for a straightforward relationship between the transmembrane electrical potential and the membrane structure, expressed here as its rigidity. The biological implications of our results are significant and of general interest. A number of events on the cellular level are known to be initiated and/or accompanied by changes in the membrane potential, like ion-transport, hormonal action, enzyme-regulation, cell differentiation and proliferation, as well as cell growth and in some cases independently concomitant modifications in the membrane structure have been observed (25,26). Vertical translocations of proteins, and availability of receptor sites in biological membranes, which can be modulated by changes in the membrane fluidity (27), as well as by electrical fields across the membrane (28), underline the interrelation between membrane structure and transmembrane potential and its general occurrence in biological systems.

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