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K/2 A NOVEL FLOW CYTOMETRIC METHOD FOR THE MEASUREMENT OF DIPOLE POTENTIAL IN LIVING CELL MEMBRANES

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Keywords: dipole potential, flow cytometry, cholesterol, 3-HF, ANEPPS

Aims: A membrane protein is exposed to three different kinds of membrane potentials, the transmembrane, surface and dipole potentials (DP). The DP originates from the preferential alignment of interfacial water dipoles and dipolar segments of phospholipids, resulting in a large (~300 mV) potential creating a significantly larger intramembrane electric field than transmembrane and surface potentials. It was shown to influence a multitude of membrane-coupled events including the gating mechanism of voltage-gated ion channels and ligand-induced clustering and activation of ErbB proteins.

Methods: Since the DP is entirely located within the plasma membrane, it is difficult to measure directly. Di-8-ANEPPS, an electrochromic, voltage sensitive fluorophore, is most commonly used to estimate its magnitude via ratiometric excitation methods using spectrofluorometry or fluorescence microscopy. However, these methods are characterized by serious drawbacks. A novel group of fluorophores, 3-hydroxyflavons (3-HF), can be used to measure the DP, due to changes in the relative intensities of their two emission bands corresponding to two molecular species interconverted to each other by excited state intramolecular proton transfer which is sensitive to the local electric field.

Results: We have developed a novel, emission ratiometric method in flow cytometry to determine changes in the DP using two 3-HF derivatives, F66 and PPZ8. We have confirmed the applicability of our method using known DP-modifying treatments. Loading the cell membrane with cholesterol increased the DP, while cholesterol extraction decreased it. Sphingolipid accumulation generated in an in vitro model of Gaucher's disease led to an increased DP.

Conclusions: In our ongoing studies, we are examining changes in the DP in diseases affecting the lipid composition of membranes with our novel method. Our preliminary results imply that the magnitude of changes in the dipole potential caused by alterations in the lipid composition of the membrane is sufficient to modify the functioning of membrane proteins.

K/3 COMPARISON OF LIGHT-TRIGGERED GENERATION OF ROS AND DIRECT EVIDENCE FOR MEMBRANE DAMAGING EFFECT OF MESOPORPHYRIN DERIVATIVES IN

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Keywords: PDT, porphyrines, liposomes, ROS, membrane-damage,

Aims: Photodynamic effect is a frequently used term in photobiology to refer to photoinduced damage of a target, requiring the simultaneous presence of light, photosensitizer and molecular oxygen. One of its applications is the photodynamic therapy which is a medical treatment based on the light-triggered generation of reactive oxygen species (ROS) by porphyrin derivatives. ROS may cause oxidative damages and, in consequence, ultimately kill cells. Besides DNA the other target of ROS are the membranes which separate internal compartments in living cells. Hence, the ability of ROS production of porphyrins in liposomes as simple models of cellular membranes is of outstanding interest.

Methods: Earlier we compared the binding parameters and locations of mesoporphyrin IX dimethyl ester (MPE) and its non esterified form, mesoporphyrin IX dihydrochloride (MPCI) in small unilamellar vesicles (SUV) made from various saturated phosphatidylcholines. In this work we used the same kinds