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Importance of Lipid Composition in the Membrane Dynamics of Urothelial Umbrella Cells

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The studies on membrane dynamics in urinary bladder umbrella cells were focused on proteins as important factors for maintaining the permeability barrier and their role as pathways modulators for the discoidal/fusiform endocytic vesicles which is one of the main features of the umbrella cells. However, our workgroup has dedicated to the study of lipid membrane composition and its biological impact in the urothelium. We first studied the lipid membrane composition of rat urothelium modified my dietary treatments differentiated in their fatty acid composition. Changes of lipid composition were related to the asymmetric unit membrane organization and permeability. Finally, we observed that the lipid composition was critically related to the intracellular pathways of discoidal/fusiform endocytic vesicles and their content. The purpose of this communication is to summarize the importance of lipids in the membrane organization and permeability of the apical plasma membrane and endocytic vesicles of umbrella cells.

Organization of the Urothelial Asymmetric Unit Membrane (AUM)

The urothelium is a specialized epithelium that covers the mucosa surface of urinary tract from the renal pelvis to the proximal urethra (1) and prevents the unregulated exchange of substances between urine and blood (2). It is composed by three layers: superficial or umbrella cells, intermediate and basal cells. *Figure 1.* We encourage on reading a recently published article of our workgroup that deals in depth with the urothelium characteristics (3).

Apical plasma membrane of umbrella cells contains specific lipids and proteins (4, 5, 6, 7). The protein constituents are the uroplakins, a group of at least five proteins including the tetraspan family members UPIa (27 kDa) and UPIb (28 kDa), and the type I single-span proteins UPII (15 kDa), UPIIIa (47 kDa) and UPIIIb (35 kDa) (8). Liang et al. by using a harsh detergent, sarkosyl, obtained from the urothelium apical plasma membrane a fraction containing plaques and hinge areas (9). The highly detergent insolubility of this luminal membrane may reflect its unusual lipid composition, which is enriched in cholesterol, phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and cerebroside (Cb), a lipid profile similar to myelin (7, 10, 11, 12). Ketterer et al. reported the presence of saturated and polyunsaturated ω-6 derivative fatty acids mainly and high levels of one eicosatrienoic (a marker of essential fatty acid deficiency) (13).

The outer leaflet of apical plasma membrane of umbrella cells appears to be twice thicker as the inner leaflet, thus forming an asymmetric unit membrane (AUM) (14). When negative staining solubilized membranes of AUM were observed by high resolution microscopy and by quick freeze-deep etch techniques, a well-developed paracrystalline array of 16 nm diameter AUM particles was observed. This paracrystalline array presents six-fold symmetry, forming a structure composed of an inner ring containing six large particles and an outer ring containing six small particles (14). Min et al. (15) suggested a possible association of the uroplakin pair Ia/II and Ib/III with the inner and outer subdomains, respectively. The images resulting from studying the electron micrographs not only revealed a striking two-dimensional (2D) organization but also the

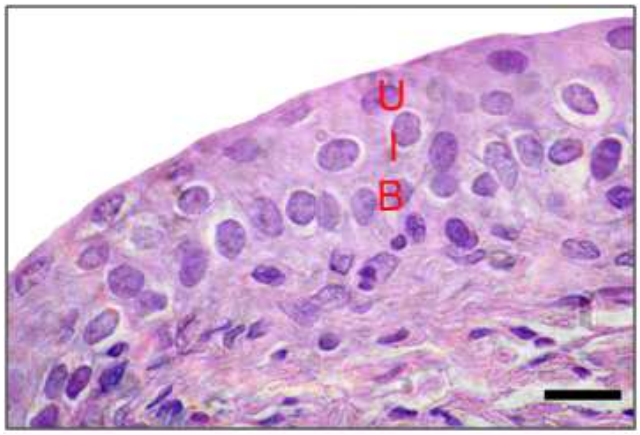

Figure 1. *urothelium from rat urinary bladder stained with Hematoxylin and Eosin. U, I and B corresponds to umbrella, intermediate and basal cells respectively. Magnification 1000x. Scale bar = 10 μm.*

presence of stain-excluding areas of a likely lipid nature. We described for the first time the dependence of AUM particles association with the membrane lipid composition. By modifying the membrane fatty acid composition by dietary treatments we observed relative changes in uroplakins dimers (12). In fact it has been shown by chemical "cross-linking" an increase of the heterodimer UPIb/UPIII and a decrease of the homodimer UPIII/UPIII in the oleic acid-derived urothelium. From those observations we inferred that a possible lipid-protein alteration may be the cause of the observed altered uroplakin dimerization (12). In agreement with those data we observed, by morphometric analyses of AUM, a significant increase of the particle size from 15 nm (control particles) to 17 nm center-to-center in oleic acid-derived particles (16). With these results we developed a hypothetical model (16) that assumes: 1) the extracellular part of the particles may be allowed to expand by changing the "twist" according to the ribbon model (14, 15), in a manner that the angle between the inner and outer subdomains of each subunit is increased thus leading to an increased total particle size. This "twist" change may be accompanied by a decreased separation between the UPIb/UPIII heterodimers as mentioned above (12, 16).

On the other hand, the destructuring effects of oleic acid derivatives on lipid bilayers were extensively studied. Oleic acid derivatives are capable to induce a marked decrease of the gel to liquid crystalline phase transition temperature, as demonstrated by Marsh (17). Also oleic acid (18:1) induces important concentration-dependent alterations in the supramolecular organization of PE derivatives, whereas the closely related fatty acids, elaidic (18:1 trans) and stearic (18:0) acid, did not (18). Oleic acid probably exerted a lateral pressure on PE fatty acids moieties, favoring a negative-curvature strain. This effect induced the formation of inverted tubular micelles, which are the basic supramolecular units of the H_{II}-phase (inverted hexagonal) lattice. This hypothesis is consistent with the marked decrease of the liquid crystalline-to-H_{II} phase transition temperature induced by oleic acid derivatives (18). The H_{II}-phase has an important role in membrane fusion events, protein control, ultrastructural organization and crystallization of membrane integral proteins (19). Curiously, we observed a high content of PE in urothelial oleic acid-derived vesicles (12) that associated to the highest content of oleic acid could induce the formation of H_{II} phase in these membranes. This hypothesis seems to have support by the increment of the bilayer disorder degree in oleic acid-derived membranes as determined by fluorescence anisotropy measurements (11).

Membrane Permeability of Endocytic Vesicles

A complete disappearance of both, the AUM and the symmetric hexagonal pattern, together with a reduced junctional complex, was observed after a direct exposure of the urothelial luminal surface to carcinogens (20). These membrane changes implicate increased permeability and less adaptability of urothelial function to mechanical stress caused by bladder volume changes (20). Moreover, knockout animals lacking the UPII or UPIIIa (major luminal surface proteins) showed and increased permeability to urea and water supporting the idea that luminal surface is a component of the permeability barrier (21, 22). However, the permeability of the endocytic vesicles was scarcely studied. To our knowledge, there are only two works where it is demonstrated that the endocytic vesicles can release their content into the cytosol (23, 24). By inducing the endocytosis of a fluorophorer and its quencher (hydroxypyrene-1,3,6-trisulfonic acid or HPTS and p-xylene-bis-pyridinium or DPX, respectively) we determined both the relative released material to the cytosol and the leakage mechanisms from urothelial endocytic vesicles differentiated in their membrane lipid composition induced by dietary treatments (23). For leakage determinations we used the requenching method developed by Wimley et al. (25) and widely extended by Ladokhin et al. (26). This method is based in a simple titration of fluorescence fraction released from the endocytic vesicles to determine the internal quenching of remaining fluorescent molecules in the interior of the vesicles. The internal quenching dependence on the fluorescence fraction released allowed us to define two possible mechanisms of leakage: a) All-or-None, where the internal quenching is independent on the released fluorescence fraction and b) Graded, where changes of the internal quenching are dependent on the released fluorescence fraction. In the first case a population of endocytic vesicles releases all of their content and others do not; in the second case the released fluorescence from the endocytic vesicles is partial (25, 26). Our results have shown not only the existence of leakage in urothelial endocytic vesicles, but also the differential mechanisms of release dependent on membrane lipid composition (23). Control and linoleic acid-derived vesicles showed a graded leakage mechanism with preferential release of cationic DPX. In the case of oleic acid-derived vesicles we could not distinguish between the All-or-None leakage mechanism and the highly graded preferential release of HPTS (23). As mentioned before, we observed a high content of PE in oleic acid-derived vesicles (12). It is known that dioleoylphosphatidylethanolamine (DOPE) promotes a non-bilayer phase which favors the formation of inverted hexagonal structures (H_{II}) that confer membrane instability (27, 28). This fact may probably be applied to oleic acid-derived vesicles where the high content of oleic acid may be associated to the increased amount of PE. Thus, this lipid interaction may

promote the appearance of non-bilayer structures and, consequently, a vesicular membrane destabilization leading to the non-differential release of its luminal content (23).

Conclusions

In this communication we summarized some of the major experimental data that reveals the importance of lipid composition in the membrane dynamics of rat urothelium. The formation of urothelial plaques and apical plasma membrane trafficking in umbrella cells are important to the formation and maintenance of the blood-urine permeability barrier. In many cases of urinary diseases, this permeability barrier is altered, and toxic compounds present in urine are internalized producing cellular toxicity. Thus, the knowing of fundamental aspects in the membrane dynamics of urothelium is the first step to understand urinary bladder´s pathologies.

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