

ANIONIC PERMEABILITY OF THE LIVER ER MEMBRANE

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ABSTRACT

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The ionic pathways present in the liver ER membrane are not known in detail. Studies using ER-derived vesicles have shown that they are permeable to Na^+ , K^+ , choline⁺ and Cl^- but less permeable to Ca^{++} and Mg^{++} . Though highly permeable to K^+ , the liver ER membrane has been postulated to lack an efficient ion conducting structure for K^+ like the K^+ , Na^+ channel in the sarcoplasmic reticulum.

InsP_3 , an intracellular second messenger can release Ca^{++} from an intracellular store of many types of cells and that store has been postulated to be the ER. An InsP_3 -gated Ca^{++} channel has been shown to exist in canine cerebellar microsomes. But, the the identity of the store in liver tissue is unclear. Though the liver rough ER-derived vesicles have been shown to release Ca^{++} when challenged with InsP_3 , the InsP_3 - binding sites copurify not with the ER marker, rather with the plasma membrane marker.

The present study was undertaken with the aim to look at the anionic, Ca^{++} and K^+ permeability pathways present in the ER membrane.

Direct current-voltage recording is a straightforward approach to delineate the ionic pathways present in any membrane. But the membrane of an intracellular organelle like the ER is not accessible to direct cellular patch recording. So, we have fused the liver rough ER-derived vesicles with a planar BLM and have made current-voltage measurements across the reconstituted BLM. In our fusion protocols the vesicles could be readily fused with a BLM by swelling them osmotically in chloride containing solutions.

Using the above experimental approach, We have found that the liver rough ER-derived vesicles possess considerable anionic permeability. The permeability to halides and other anions follows the sequence : $\text{SCN}^- > \text{I}^- > \text{Br}^- > \text{Cl}^- \gg \text{gluconate}^-$, suggesting that the chloride channels have low field-strength sites. It can be pharmacologically dissected to Zn^{++} -sensitive and DIDS-sensitive types. DIDS blocked the chloride permeability from the cytoplasmic side of the ER. No InsP_3 -gated Ca^{++} channels, ryanodine-sensitive Ca^{++} channels and K^+ channel were found in the liver rough ER membrane.

DEDICATION

**To my parents, brothers and sisters
and my wife Moni and my son Arnav.**

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INTRODUCTION

All cells contain an endoplasmic reticulum (ER). Though highly convoluted, the ER membrane is thought to form a single continuous sheet, enclosing a single sac. The ER plays a central role in the biosynthesis of macromolecules used to construct other cellular organelles. Lipids, proteins and complex carbohydrates destined for transportation to the Golgi apparatus, to the plasma membrane, to the lysosome, or to the cell exterior are all synthesized in association with the ER. Two functionally distinct regions of the ER can be easily identified in some cells: the rough ER and the smooth ER. The rough ER is studded with ribosomes on the cytoplasmic side of the membrane. Numerous morphological investigations have demonstrated rough ER and smooth ER to be in direct physical continuity; rough ER is thought to give rise to smooth ER by a process of cisternal "budding." Physical disruption of the cells (homogenization) results in the conversion of both forms of ER into spherical vesicles which can be isolated as the microsomal fraction by differential centrifugation.

Several permeation systems for ions and small solutes are present within the reticulum structures of cells. Three transport proteins, T_1 , T_2 and T_3 are required to enable glucose-6-phosphate, phosphate (and pyrophosphate), and glucose to respectively cross the ER membrane (1). Other biologically relevant solutes and ions that cross the ER membrane include D-glucose, L-glucose, L-leucine, choline⁺, K⁺, Na⁺ and Cl⁻ (2). Meissner et al. (2) found that there are two types of liver microsomes (designated as types A and B) with differing permeabilities to glucose and other small molecules. About 70 percent of the

microsomes (type A) are permeable to D-glucose, L-glucose, 2-deoxy-D-glucose, D-mannose, D-mannitol, uridine, glycine, L-leucine, choline⁺, TRIS⁺, Rb⁺, K⁺, Na⁺, and Cl⁻. All of the above solutes, except Cl⁻, pass with a comparatively slow rate in the remaining 30 percent type B vesicles. Type A and B vesicles are similar in that both are essentially impermeable to sucrose, yet permeable to Cl⁻. By making membrane potential measurements with a fluorescent dye probe, Meissner et al. found that a significant fraction of ER vesicles were more permeable to TRIS⁺ than to Ca⁺⁺ or Mg⁺⁺. They also made another important observation that, despite their preferential permeability to K⁺, a majority of liver microsomes lack an efficient ion-conducting structure for K⁺, such as the K⁺, Na⁺ channel which renders above two-thirds of the SR vesicles highly permeable to K⁺. Treatment with the anion transport inhibitor 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) lowered the permeability of type A vesicles to several uncharged and negatively charged solutes, including D-glucose and gluconate⁻. Based on their results, they suggested that a large fraction of liver microsomes is rendered permeable to various biologically relevant solutes and ions, perhaps through the presence of one or more channels with a maximum diameter of approximately 7-8 Å which select(s) against solutes on the basis of their size and charge.

The role of ER in sequestering Ca⁺⁺ has been well recognized. Out of the two major intracellular organelles-i.e., the mitochondria and the ER, now there is general agreement, largely through the application of electron probe x-ray microanalysis to fast frozen tissue, that mitochondria contain little Ca⁺⁺, compatible with the regulation of mitochondrial enzymes but can sequester massive amounts, should the cytosolic Ca⁺⁺ begin to rise and that, despite its relatively small Ca⁺⁺-binding capacity, the ER looks the stronger candidate for a high affinity physiologically relevant Ca⁺⁺ store (3). A Ca⁺⁺-ATPase exists in

the ER membrane. The rat liver microsomal Ca^{++} -ATPase has been purified (4). Its molecular weight is 107 kDa and antiserum raised against the 100 kDa sarcoplasmic reticulum (SR) Ca^{++} -ATPase cross-reacted with it. A major Ca^{++} -binding protein, calreticulin (analogous to calsequestrin), has been shown to be present in the smooth muscle SR and liver ER (5). In this connection it should be borne in mind that SR, a specialized derivative of ER has long been known to be the intracellular Ca^{++} store in skeletal and cardiac muscle.

The rise to prominence of the ER has brought new ideas about Ca^{++} mobilization and, together with studies on the SR, a clear picture is beginning to emerge about the Ca^{++} sequestration and release processes and their control in these systems. A major step in this direction has been the discovery of inositol 1,4,5-trisphosphate (InsP_3) as an intracellular second messenger (6) and its role in releasing Ca^{++} from the ER of many types of cells (7). An inositol lipid located within the plasma membrane is the precursor used by the receptor mechanism to release InsP_3 to the cytosol, leaving 1,2-diacyl glycerol (DAG) within the plane of the membrane. Conceptually, this theory became very attractive, since, in one step, it provided a link between membrane receptors and release of Ca^{++} from a major intracellular store. Consistent with its role as a second messenger, the increase in the level of InsP_3 was found to precede the onset of Ca^{++} -dependent events in blowfly salivary gland (8) and in neutrophils (9). The transduction unit within the plasma membrane consists of three main components: 1) a receptor that detects the incoming signal; 2) a G protein that serves to couple the receptor to the third component; and 3) a phosphodiesterase responsible for cleaving the lipid precursor.

Ca^{++} is constantly cycling due to passive efflux and active influx across the ER membrane, and all the available evidence points to InsP_3 acting to stimulate the passive efflux component while having no effect on the pump.