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13. ABSTRACT *(Maximum 200 words)*

Mechanically sensitive ion channels have been proposed to respond to membrane tension. We have developed methods to measure the membrane tension in a patch and have examine the stress strain relationship. The results show that, in general, tension is the critical variable. However, we have also found one channel in glial cells whose gating is additionally dependent upon the curvature of the membrane. Stresses in a patch caused by suction lead to lipid flow along the wall of the pipette, but the flow is constrained by the highly extensible cytoskeleton whose area elastic constant is ca. 50dyn/cm.

We developed tools to use high voltage electron microscopy to study the structure of patches and have characterized the placement of cytoskeleton, lipids and receptors in a variety of preparations. We also developed a new algorithm to align projections for tomography in order to examine the three dimensional structure of patches.

We have demonstrated mechanically induced release of calcium via stretch activated ion channels in heart cells extending work on the molecular level to the whole cell level.

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Effect of Cytoskeletal Reagents on Stretch Activated Ion Channels

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Problem Studied:

What makes cells mechanically sensitive? This work examined the mechanical properties of patch clamped cell membranes with regard to both structure, stress-strain relationships and properties of the mechano-electric transduction channels.

Major results:

We have found that patch clamped membranes always are formed with the cytoskeleton attached. this has been verified by 2D and 3D electron microscopy. We have developed and published procedures for making such images. Working with light microscopy, we have found that the cytoskeletal structure of patches is highly variable. Sometimes the cytoskeleton sticks in a compact layer to the membrane and there is little Brownian motion of entrained particles. In other cases the cytoplasm is disrupted and particles diffuse readily. The variability of the structures accounts, at least in part, for the variability of stretch induced responses in cells.

By combining electrophysiology with light microscopy of patches we have been able to measure the elasticity of membranes and to clearly separate the properties of lipids from those of the cytoskeleton. In skeletal muscle cells the cytoskeleton has an area elastic constant of about 50dyn/cm and this value is insensitive to treatment with cytochalasin suggesting that actin is not tension bearing in this membrane. In heart cells, on the other hand, the elasticity decreases to 25dyn/cm with cytochalasin. We were able to show that under stress, the lipids in a membrane patch are free to flow along the walls of the pipette. We were able to show that a major source of error in the measurement of stretch activated channel sensitivity can be removed by using as the independent variable tension rather than applied pressure.

We studied mechanical transduction in a variety of cells types and found mechanically sensitive channel in the lateral walls of auditory hair cells from the guinea pig. These channels are not the normal transducers responsible for the major sensory activity but may play a role in tuning. These channels may explain why hair cells will contract when a frequency modulated water jet is applied to the cell body.

We found in glial cells that there are multiple types of transduction channels and one of them has the unique property of only responding when the membrane is curved toward the cytoplasm. This is the first observation of such a curvature sensitive channel.

On a cellular level we have shown that mechanical stimulation of heart cells can cause the release of intracellular calcium. The mechanism of this coupling probably involves stretch activated ion channels since the response is blocked by low extracellular calcium, blockage of the channels with Gd ions or suppression of channel expression by omission of growth factors from the culture medium.

Participating Scientific Personnel:

Dr. Frederick Sachs, Dr. Charles Bowman, Dr. Masahiro Sokabe, Dr. Wade Sigurdson, Dr. Zhonqi Jing, Dr. R.J. Salvi, Dr. J.P. Ding, Dr. A. Ruknudin, Dr. X.C. Yang (obtained his PhD during the project),

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