GIGAOHM SEALS TO MEMBRANE EVAGINATIONS OF *ILYANASSA OBSELETA* EGGS

David Busath, Andrew Campbell, and Greg Hemsley
Section of Physiology and Biophysics
Brown University, Providence, RI 02912

We have attepted to implement the patch-clamp technique (Hamill et al., Pflugers Arch., 391: 85-100, 1981) on eggs from the marine snail *Ilyanassa obsoleta*. Freshly laid, fertilized eggs were rinsed in ASW (490mM NaCl, 10mM KCl, 50mM $MgCl_2$, 10mM $CaCl_2$, 5mM HEPES, pH 7.0) and used during the first through fourth polar lobe stages. Patch clamp pipettes were filled with 250mM NaCl, 250mM KCl, 50mM $MgCl_2$, 10mM $CaCl_2$, 5mM HEPES, at pH 7.0. The bath temperature was 23 - 24° C. The resting potential under these conditions was measured to be between -65 and -75 mV.

Using untreated eggs, seal formation was not possible (all efforts yielded resistances below 50 MOhms). Various locations on the cell were tested at each of the stages of cell development. Digestion of the eggs in Protease XIV (Sigma, 2 mg/ml in ASW for 20 mins or 2 mg/ml in Choline ASW - 490mM Choline Chloride instead of NaCl - for 2 hrs); Collagenase (Sigma, 2 mg/ml in ASW for 20 mins); or Collagenase and Hyaluronidase (Sigma, 1 mg/ml each in ASW for 20 mins) did not facilitate seal formation. However, gigaohm seals did form with cell membrane evaginations or precipitation membranes (Heilbrunn, *The Colloid Chemistry of Protoplasm*, Berlin, 1928, p215-232). These were induced by impaling the eggs with a broken micropipette. These small bleb-like structures were distinctive in that they contained none of the large cytoplasmic granules found throughout the oocyte and they had a smooth surface usually free of the microvilli characteristic of some of the developmental stages of the oocyte. They commonly formed 30 - 90 mins after impalement and were often localized at the base of the impalement-induced lobe structures. The resistances of seals to these evaginations ranged from 2 to 20 GOhms.

In some patches we were able to observe single channels. The membrane potential was stepped from a holding potential of 0 mV to various test voltages. The holding period and test period were each 1.1 sec. Capacitative currents were removed by adding the current samples obtained during the test step to those from the return step to the holding potential. The figure below shows the corrected current for the entire test period for each of three test potentials, -170, -180 and -190 mV (top to bottom). In this example the patch had been excised from the cell after seal formation so that the cytoplasmic surface was exposed to the bath ASW. The horizontal bar represents 100 msec, and the vertical bar represents 10 pA. At a membrane potential of -160 mV, the single channel current was 7.0 pA. The channel selectivity and identity remain uncertain. The results of this study demonstrate that the identification and study of snail egg membrane channels are possible using the patch-clamp technique.