Oocyte Biophysics

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Introduction

To study electrophysiological properties of a cell, the cell has to be integrated into an electrical circuit. Due to the small size of cells, this circuit has to be very sensitive, and has to avoid drawing large currents, which would alduterate the measurement significantly. Another problem is the connection of the cytoplasm to the circuit without producing a bypass between intra- and extracellular space. The appropriate method that solves these problems is voltage clamping. Voltage clamping can be used to determine voltage current relationships in clamping the cell to a certain voltage and measuring the current that has to be injected to maintain this voltage. From this, the membrane resistance can be calculated. Furthermore, the existence of voltage gated channels can be investigated from the $IV-$relationship. Another application of voltage clamping is the examination of effects of signaling molecules on physiological properties of the cell, in particular on channel proteins in the membrane.
In this lab, voltage clamping was used to study *Xenopus* oocytes. These cells have the advantage to be very large (1mm in diameter), which makes it easier to clamp the cells using sharp glass electrodes. The membrane resistance was determined as the slope of the - linear - current voltage curve. In a next step, mRNA coding for voltage gated potassium channels was injected. Two and four days after injection, voltage and current were measured. This is a common technique to check for a successful expression of this kind of mRNA. A last experiments examined the lysophosphatidic acid (LPA) signal transduction passway, that induces Cl^--oscillations.

Materials & Methods

Very sharp and thin glas electrodes, filled with 2M KCl as electrolyte, were poked inside the vegetal (light) pole of the cell, producing a high-resistance seal between membrane and electrode. The extremly small inner diameter of the electrode tip prevented mixing of the electrolyte and the cytoplasm. Apart from that, the electrodes had a resistance between 0.5 and 3 MΩ due to the small tip. The electrodes were connected to high resistance op-amps, preventing too large currents drawn from the cell. One of the electrodes was used to set the desired clamp-voltage ($V_{command}$), while the other electrode supplied and measured the current I necessary to maintain this voltage (Fig.1).
The electrodes were inserted in the oocyte while measuring the voltage relative to

extracellular space (no clamping). When both electrodes showed a resting potential between -20 and -50mV, the cell was clamped to the desired voltage, using maximum and fast gain. The voltage clamping was realized using the *OC-725C Oocyte Clamp*, the current output was low-pass filtered. The data were acquired on a computer using the software *winwcp* and displayed on an oscilloscope. The cell-chamber was perfused during the measurements with Ringer solution (for formulas for solutions see [1]).

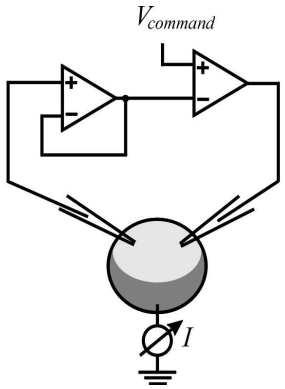

Fig.1 Voltage clamp circuit

For the $IV-$characteristic, the cell was clamped successively to voltages between -80 and -35mV with increments of 5mV. Between adjacent measurements, the cell was clamped back to its resting potential. $IV-$curves were obtained using average values for the current and voltage after equilibrium was reached (after approximately 0.4sec, see Fig.2). The slope of the $IV-$curve is the conductance of the cell membrane. Since the oocytes have a spherical shape, the membrane conductance C_{exp} can be used to estimate the diameter of the cell, if a specific membrance conductivity of $c = 0.3mS/cm$ is assumed: $d = 2 \cdot \sqrt{\frac{C_{exp}}{4\pi c}}$.
In the next step, mRNA coding for voltage gated potassium channels was injected into cells (with help from Dr. Quoc-Thang Nguyen). The expression should be completed after not more than 48 hours after injection. Two and four days after injection $IV-$curves were measured. Since the resting potential of the channels is -90mV, the voltage range was changed for this experiment to -130 to -40mV with increments of 10mV.
The last experiment concerned LPA induced Cl^--oscillations. LPA binds on the LPA-receptor, that is present on the surface of the membrane of *Xenopus* oocytes. This induces a G-protein coupled mechanism inside the cell, that eventually leads to an increase of the intracellular IP_3. IP_3 induces release of Ca^{2+} from the Endoplasmatic Reticulum that activates membrane bound Cl^- channels. Due to the fact that there are three different kinds of Cl^- currents, both inward and outward, that are acting on different time scales, this results in an oscillating behaviour of the overall

chloride current of a voltage clamped cell [2]. As source for LPA was goat serum used. It was diluted 1:1000 with Ringer solution and the cell was perfused with this solution instead of Ringer during the recording. Between adjacent recordings, the cell was perfused with Ringer for at least 15min. During the measurement, the cell was clamped to a constant voltage of -40mV while measuring the current necessary to maintain this voltage.

Results & Discussion

The voltage-current curve (Fig.3) is linear over the measured range. From the slope of the regression line, a membrane resistance of $R_{exp} = 60k\Omega$ was obtained. From this value, a cell diameter of 1.3mm was calculated, which is a reasonable value (standard value for *Xenopus* oocytes: 1mm).
The $IV-$curve for the cells with mRNA injected is shown in Fig. 5. These data were obtained two days after injection, the data obtained four days after injection looked essiantially the same (not shown). Obviuosly, the relation between current and voltage is linear over the full measured voltage-range. Similar data were obtained for seven different cells. The only explanation I can think of is unsuccessful expression of the mRNA. The deviation of the $IV-$curve from linear behaviour for large voltage (-130mV) is probably due to the extremely high electric field build up over the membrane: The effect of electroporation produces small pores in the membrane, increasing the conductance of the membrane.
For the last experiment, the LPA induced Cl^--oscillations, the recorded data are shown in Fig.6a. For analysis of the data, the power spectrum for a moving window of width 5 sec was calculated and is shown as a color plot (Fig. 6b). The x-axis is time, the y-axis frequency and the colors code for the power of the different frequencies (blue: low power, yellow & red: high power). Especially in the region of rather regular oscillations (between 10 and 30 sec on time axis), a strong frequency of 1Hz can be seen. During the lab was it not possible to reproduce these data, an error analysis is hence not possible.
During the experiments, a number of major problems appeared and had to be taken care of. One of the major concerns was the condition of the oocytes. Only in healthy and "young" (less than four days) oocytes the electrodes could be inserted without damaging or killing the cell. Apart from that, the obtained resting potential depended on the condition of the cell, old cells gave resting potentials of only about -10 to -15mV. Even if this should not effect the membrane resistance, for instance, it increases the error of the measurement. Possibly, it could also lead to a decreased resistance due to larger leak currents. The quality of the electrodes was another important factor for a successful experiment: Unless the elecrodes had thin tips and no air bubbles in the tip, recordings could not be obtained. Learning the skill to poke the oocytes succesfully took the biggest part of the time during the lab.

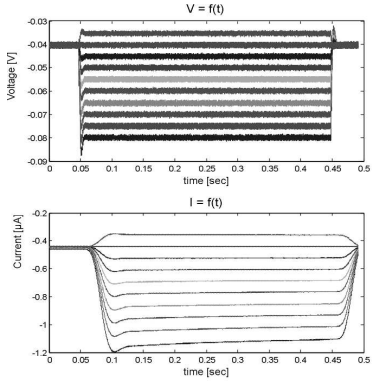

Fig.2: I and V vs. time

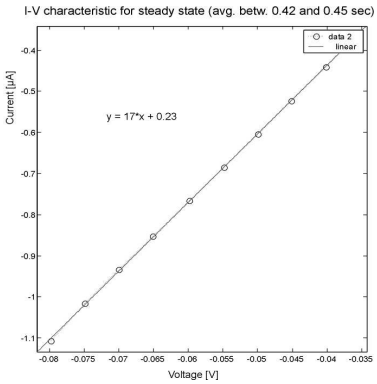

Fig.3: $IV-$characteristic for *Xenopus* oocyte

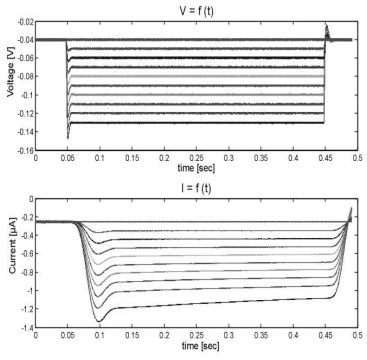

Fig.4: $I(t)\&V(t)$ after injection of mRNA

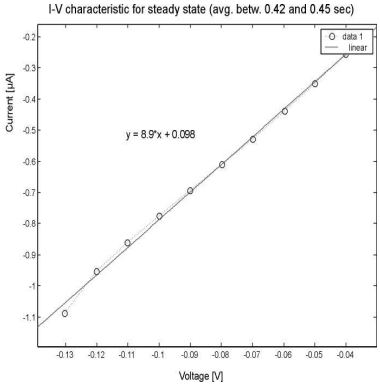

Fig.5: $I(V)$ after mRNA-injection

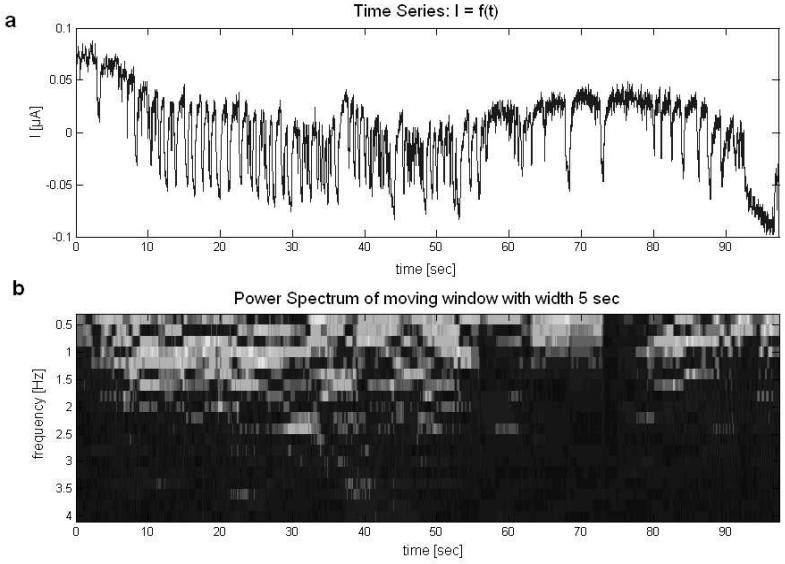

Fig.6: LPA induced Cl^- oscillations in *Xenopus* oocytes

Conclusion

The described voltage clamp experiment was used to determine the membrane resistance of *Xenopus* oocytes (60kΩ). With a standard conductance for biomembranes, the radius of the cell was calculated from this resistance. In examining the current voltage relationship of cells with mRNA coding for potassium channels, we showed that the expression of this mRNA was not successful. Finally, LPA induced Chloride oscillations were recorded and a base frequency of 1Hz was determined from the Power spectrum. Future work could include a repition of the mRNA injection experiment and examination of chloride oscillations induced by IP_3 injected into the cell.

References

[1] Bloogood, B. et al.: Lab Manual for Oocyte Biophysics, 2001.
[2] Kuruma, A. & Hartzell, H.C.: Dynamics of calcium regulation of chloride currents in *Xenopus* oocytes. *Am J Physiol.* 1999.