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LPA Induced Cl^- Oscillations in *Xenopus* Oocytes

Nini Huynh
David Marciano
Chisa Suzuki

If only we hadn't poked these oocytes, how cute would it be!

INTRODUCTION

Electrophysiology in the *Xenopus* oocyte began in 1982 when scientists realized that they could inject mRNA into oocytes and express functional ion channels and receptors. Since this time scientists have utilized this important technique to study several aspects of the structure and function of ion channels and receptors. These include:

- (1) Analyzing the properties of mutated channels as a means to understand the structure-function relationships in ion channels.
- (2) Studying the post-translational processing and the assembly of multisubunit channels and receptors.
- (3) Comparing the properties of channels from various tissues expressed in a common environment.
- (4) Examining the modulation of channel and receptor function by various second messenger systems.
- (5) Analyzing various aspects of receptor-effector coupling.
- (6) Functional screening of cloned genes that encode channels and receptors.

Source: www.axon.com

Most of these experiments require electrophysiological recording from oocytes. It is therefore important to have a basic understanding of the oocytes and the techniques used to record from them.

The Xenopus Oocytes:

Xenopus oocytes are the precursor to frog eggs, the difference being that they have not undergone the proper hormonal stimulation to mature. The oocyte is a large cell, with a diameter of ~1-1.2mm making them ideal for electrophysiological recordings. The spherical structure (fig 1.) of the oocyte is characterized by two distinct hemispheres; the animal pole (dark) and the vegetal pole (light), the animal pole being where the nucleus is located.

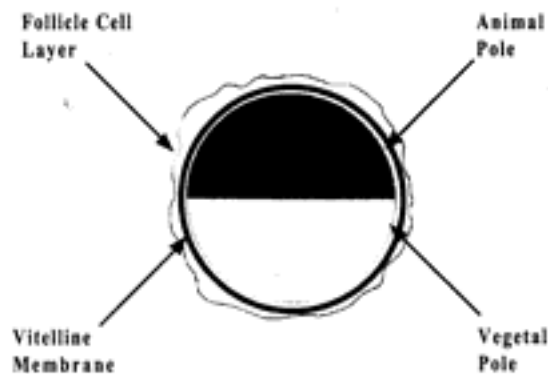


Fig. 1: www.axon.com

Another important structural component of the oocyte is the follicle cell layer. The follicle cell layer is a source of many potential problems, the first being one of

practicality. It is harder to poke an oocyte when it has its follicle cell layer attached. Another potential problem is that there is electrical coupling between cells in the follicle cell layer, which can be mistaken as electrical events occurring inside the oocyte. For these reasons, it is important to remove the follicle cell layer before poking the oocyte (see lab manual).

AMENDED PROCEDURE

We closely followed the procedure describe in the *2001 Lab Manual for Oocyte Biophysics* making only a few modifications. Our goal was to introduce goat serum containing LPA into the oocytes bath and observe Cl^- oscillations. A detailed explanation of the cascade that produces these oscillations is to follow in the next section.

One departure from the given procedure was the concentration of goat serum that we exposed the oocytes to. Our dilution ratio was 1:10 while last years procedure called for 1:1000. A detailed discussion of the effect this change in serum concentration has on the Cl^- oscillations is to follow. Another change to the given procedure was the technique used to introduce the serum. The lab manual called for the oocytes to be perfused with a serum/saline mixture. Instead, we setup a 3rd micro injector with a blunt tipped pipette. We then aimed the pipette (full of serum) a few diameters upstream of the oocytes keeping the perfusion on. A blast of 100 mseconds seemed to be an adequate amount of serum to illicit an oscillatory response. After the oocyte was exposed to the serum we recorded for 800 seconds getting oscillations after ~400 seconds.

BACKGROUND

Ca^{2+} mobilization system due to intracellular chemical reaction cascade in *Xenopus* Oocytes can be induced by acetylcholine (ACh) or serum (Fernhout, Dijcks, Moonlenaar, and Ruigt, 1992). Both ACh and Lysophosphatidic acid (LPA), found in serum, causes signaling pathways which increases intracellular concentrations of inositol-1,4,5-triphosphate (IP_3). IP_3 binds to its surface receptor on the endoplasmic reticulum (ER) which results in a rapid release of Ca^{2+} from the ER (explained in more detail in *2001 Lab Manual for Oocyte Biophysics*). This is then followed by an influx of extracellular Ca^{2+} via a plasma membrane signal activated by Ca^{2+} depletion in the ER (Callamaras and Parker, 2000). This increase in intracellular $[\text{Ca}^{2+}]$ opens Ca^{2+} dependant Cl^- and K^+ ion channels (Dascal, Gillo and Lass 1984). Further increase of Ca^{2+} inhibits Ca^{2+} release from the ER. Superposition of Cl^- and K^+ influx and Ca^{2+} feedback causes current oscillation, which can be observed using the voltage clamping technique.

Despite the considerable variation of oocyte response among donors and batch of collagenase used (Dascal, Landau and Lass 1983), there is some similarity in the character of oscillation that can be observed..

The shape of response to ACh can be analyzed as four components D_1 , D_2 , H and F (D_1 and H found less variant with various conditions in oocyte than D_2 and F). Early, fast inward current D_1 and late, slow inward current D_2 both result from increase in Cl^- conductance due to Ca^{2+} release and influx. The fluctuations F are a result of individual Cl^- channel events (Dascal, Landau and Lass 1983). H is a long lasting outward current due to an increase in K^+ conductance (Dascal, Gillo and Lass 1984).

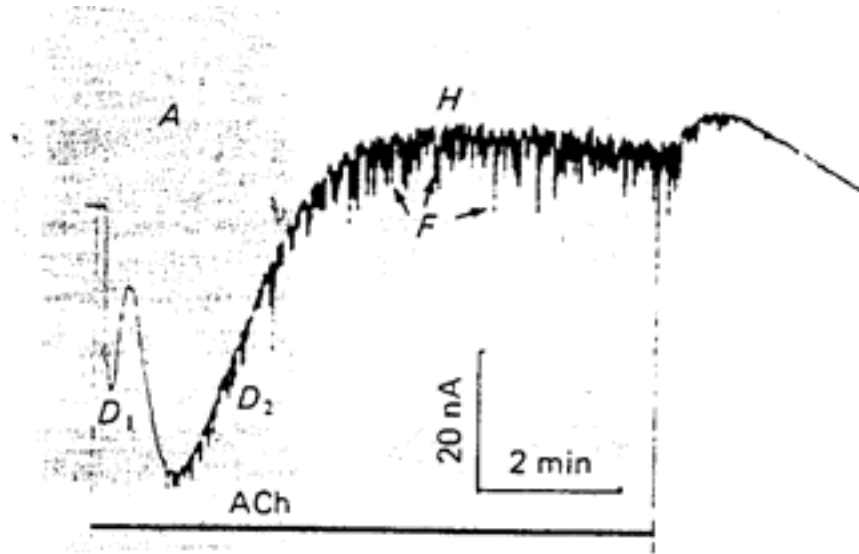


Fig 2: Four muscarinic responses to ACh in a voltage clamped *Xenopus* oocyte. (Dascal, Landau and Lass 1984)

D_1 is the fast inward Cl^- current, due to Ca^{2+} release from the ER. The Ca^{2+} will then bind to the Cl^- channel, activating it by opening the channel, and thus allowing Cl^- to enter. The stored Ca^{2+} by the ER, when released, also induces extracellular Ca^{2+} to enter the cell. This influx of extracellular Ca^{2+} raises the intracellular $[\text{Ca}^{2+}]$ high enough that it will bind to more chloride channels and so more Cl^- will enter the cell. This gives the second peak D_2 .

Since there are multiple types of Ca^{2+} dependent Cl^- channels with different affinities for Ca^{2+} (Tigyi, Dyer, Matute and Miledi, 1989), it makes sense that we see fluctuations, indicated by the F component. Also, since influx of extracellular Ca^{2+} is coupled to the ER depletion of Ca^{2+} stores, this may be further explanation of the fluctuations. When intracellular $[\text{Ca}^{2+}]$ reaches a threshold, IP_3 is inhibited from binding to the Ca^{2+} channel located on the ER, which controls the release of Ca^{2+} from storage. As intracellular $[\text{Ca}^{2+}]$ decreases, it triggers IP_3 to bind to the ER, releasing Ca^{2+} from the stores again. This continuous cycle explains the oscillation.

Finally, the smooth H component is comprised of the slow, long lasting outward K^+ current. The increase in intracellular $[\text{Ca}^{2+}]$ opens Ca^{2+} dependant Cl^- channels as well as K^+ channels. This allows for K^+ to enter the cell, producing a slow inward current. The K^+ channel is believed to be open throughout the entire oscillation, however during D_1 and D_2 , Cl^- influx dominates the K^+ contribution

As mentioned previously, LPA induced oscillations have strong dependence on the concentration of the serum. This correlation is depicted in Fig 3. As the serum concentration increases, the amplitude of induced current increases exponentially.

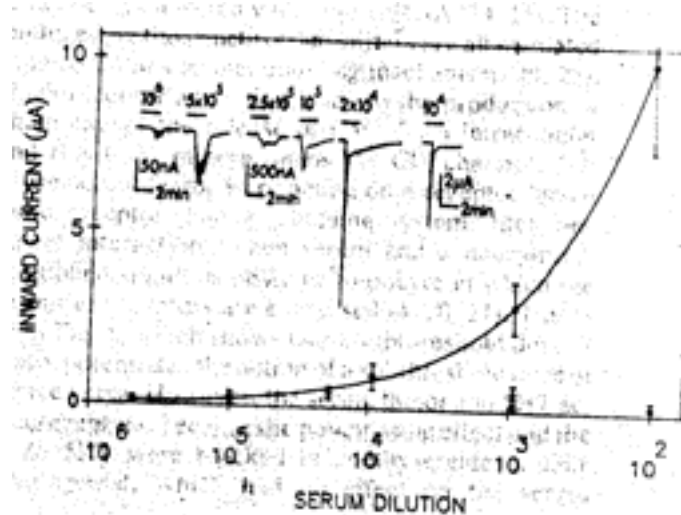


Fig 3 Dose response relation for serum-induced currents (Tigyi, Dyer, Matute and Miledi 1990)

At high serum concentrations, the current response is similar to that of ACh , characterized by a large initial peak, followed by a transient secondary peak with slow decay (Tigyi, Dyer, Matute and Miledi 1990).

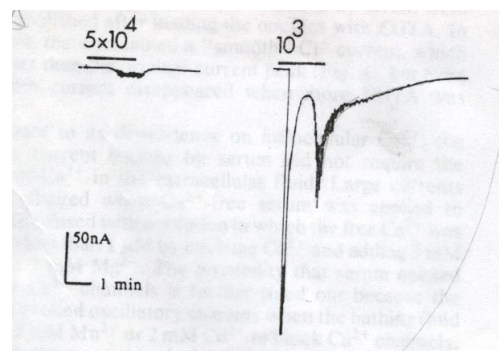


Fig 4: Membrane currents induced by normal rabbit serum in a defolliculated *Xenopus oocyte* (Tigyi, Dyer, Matute and Miledi 1990)

RESULTS

The following graphs are current recordings of an oocyte that produced what we believe to be calcium induced chloride oscillations. The oscillations had a period of ~1 minute and an amplitude of ~20 mA.

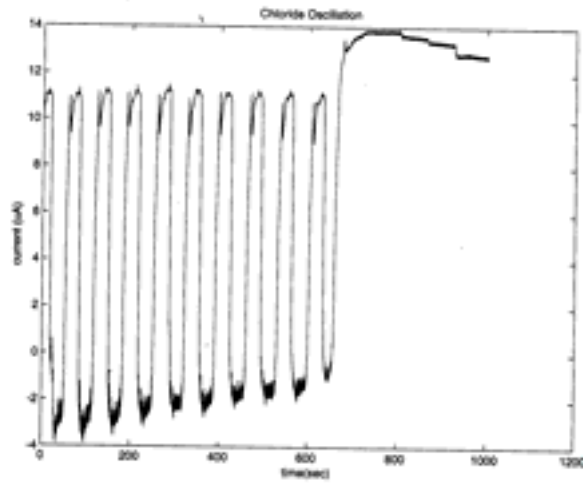


Fig 5: Calcium induced chloride oscillations (5/21/2002)

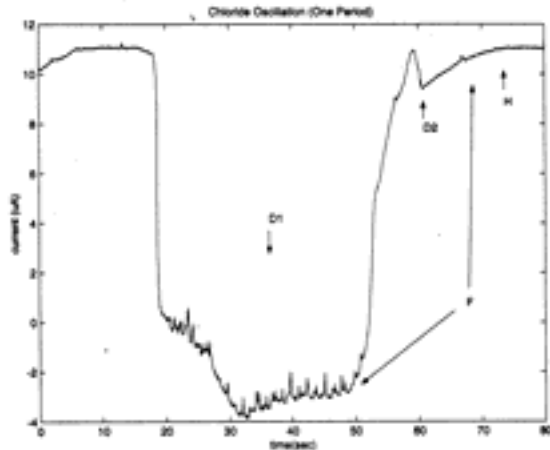


Figure 6: Single Chloride Oscillation (5/21/2002)

ANALYSIS

In comparing our graph to those of published works, we find that there were many similarities. For example, the shape of our graph for high concentrations of serum included a large initial peak, followed by a slow transient current, current fluctuations, and finally, a slow smooth component. We illustrated these similarities between oscillatory patterns in Fig 6.

Our period of ~1 minute further supports our data when compared to Fig 4. The amplitude of our induced current favorably agreed with the predicted value. An

extrapolation of the graph in Fig 3 would indicate that a serum dilution of 10, would correspond to induced current of ~20 mA, which was indeed what we observed in our oocyte.

We observed several discrepancies between our results and published data. Even though the shape of our graph had the four components, D_1 in our graph lasted longer than the one in Fig 4. Also, our fluctuations F began at D_1 , instead of at D_2 . Possible explanation of these discrepancies may stem from the variation in oocyte vitality and experimental conditions. Fig 4 used a serum dilution of 10^3 while our protocol required a dilution of 10, which may have altered the shape of D_1 . Furthermore, $[Ca^{2+}]$ in our frog ringer was different from the published works, which may have effected the D_2 response. Also, we clamped our oocyte at $-70mV$ while the experiment showing Fig 4 clamped their oocyte at $-60mV$. Since clamping the cell at a certain voltage made sure it is away from the equilibrium potentials of Na^+ , K^+ , Cl^- , and Ca^{2+} , the difference in clamping potentials may have had an effect on channels. Further investigation of these procedural discrepancies may be interesting for future study.

DISCUSSION

When we finally saw what we thought were Ca^{2+} induced Cl^- oscillations, we wanted to compare them to last year's graph. Since we were not able to find any similarities, we went to confirm with published works. In analyzing our results with those of published papers, we are happy to find that we did finally have an oscillation from the poor numerous oocytes we diligently poked.

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