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Acetylcholine-Induced Membrane-Potential Oscillations in Xenopus laevis Oocytes

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ACETYLCHOLINE-INDUCED MEMBRANE-POTENTIAL OSCILLATIONS IN XENOPUS LAEVIS OOCYTES

by

Corinne N. Grant

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Biology)

The Honors College

University of Maine

May 2009

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Thesis Advisor: Dr. Harold (Dusty) Dowse

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Over the past several years, amphibian species have shown a sharp decline in population numbers. Many factors are believed to play a role in the loss of amphibian species. One of particular interest is the increase in ultraviolet (UV) radiation reaching Earth's surface. To estimate the health of a species, an assay needed to be developed. Health can be determined by measuring the ion channel functionality of the amphibian oocyte membrane. To develop this assay, we used acetylcholine to induce a membrane-potential oscillation in *Xenopus laevis* oocytes. Several studies have studied the membrane-potential oscillation of calcium-dependent chloride channels activated by acetylcholine, but no other study has used modern analytical techniques to determine the rhythmicity of oscillation. This study determined the period of the oscillation to be from 30 to 110 seconds in length. Future studies can be conducted using this protocol to determine the effect of ultraviolet radiation on these induced oscillations. A few studies have determined that UV radiation can be used to depolarize or hyperpolarize cellular membranes (Horn et al., 2000). This involves the opening or closing of voltage-gated ion channels and potentially affect early frog development and increase mortality.

ACKNOWLEDGMENTS

I would like to thank Dr. Leonard Kass for his assistance in using the Dagan amplifier and BIOPAC recording device. Dr. Kass helped tremendously in trying to develop a monitoring system, which operates with minimal interference, to record the action of the ion channels. He also took time to ask pointed questions and reviewed cell membrane electrophysiology information.

I would also like to thank my advisor, Dr. Dusty Dowse, for all of his help. I have learned a tremendous amount this semester from working in his lab. Dusty has spent endless hours with me analyzing data and editing results. He taught me to understand confusing processes and made sure I was not overwhelmed.

I owe thanks as well to Dr. J. Malcolm Shick for suggesting the idea of using oocytes under ultraviolet radiation to observe ion channel function. Hopefully, future research will take a closer look into this concept, and will be able to use the assay we have created to study its effects.

Finally, thanks also to Sharon Ashworth and Mark Haggerty for serving on my committee. Sharon is friendly and easy to talk with, which helped reduce the stress of presenting my thesis. Mark has been, and continues to be, one of my favorite professors from the Honors College. His inquiries about my topic have inspired me to look more closely at my research and gave me the ability to explain it more efficiently.

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INTRODUCTION

Since the 1980s, researchers have noticed a dramatic decline in amphibian populations. Amphibians can be used as an indicator of overall environmental health, making the amphibian decline a huge public concern (Collins and Storfer, 2003; Blaustein and Belden, 2003). Stuart et al. (2004) found that amphibians are actually more threatened and are declining more rapidly than either birds or mammals. They also found that 43.2% of amphibian species are experiencing population decreases, while 0.5% are increasing, 27.2% are stable, and 29.1% have an unknown trend. This raises several questions about the reasons behind this significant decline.

Many factors such as over-exploitation of land, environmental contaminants, and emerging infectious diseases are believed to play a role in this decline of amphibian populations (Blaustein et al., 2003; Collins and Storfer, 2003). One of particular interest is the increase in ultraviolet radiation reaching the surface of the earth. Several researchers argue that UV radiation has no role in the decline of amphibians, while others maintain that it may play a role in concert with other factors. Of the latter group, Blaustein and Kiesecker (2002) argue that ultraviolet radiation has a direct effect on the loss of amphibians. "UV-B radiation can kill amphibians directly, cause sublethal effects or it can work in concert with contaminants, pathogens, or with changes in climate to adversely affect amphibians."

One way to examine the overall health of an amphibian is to observe the health of the oocytes, or immature ova, from an adult female frog. By studying the functionality of its cell-surface ion channels, one aspect of the health of the oocyte can be estimated. Ion

pumps are vital for the survival of the oocyte because of their ability to maintain a normal resting potential and cell functions. Ultraviolet radiation could play a role in the condition of these oocyte ion channels. Inactivation gates could be selectively trapped in the open or closed position by ultraviolet radiation at either a hyperpolarized or depolarized voltage (Horn et al., 2000). This could harm the maturation of the oocyte, its fertilization, and future embryonic development (Hille, 2001).

Ion Channels

More than 3 billion years ago, replicating forms became enveloped by a lipid layer, which functioned to separate cellular components from the environment, thereby forming a primitive cell. This was an advantage for the cell in order to protect its vital parts, but it prevented the movement of necessary ionized nutrients and waste in and out of the cell. Thus, cells needed to develop a new transport mechanism across the membrane. Prokaryotes, such as gram-negative bacteria, have simple channels and transporters through which metabolites pass (Hille, 2001). More developed life forms, such as eukaryotes, developed a more selective transport system of ion channels and transporters. Ion channels are macromolecule pores found on all viable cell membranes and are the crucial elements for excitable membranes of the nerve and muscular systems (Hille, 2001; Tabone, 2000).

Ohm's law is central to understanding the movement of ions through these channels. This law relates current, voltage, and conductance. All matter is made up of charged particles. Any flow of these charged particles is referred to as current. The size of the current is determined by the potential difference between the electrodes, which

measure current and voltage, and the electrical conductance of the solution between them. The electrical potential difference, or voltage, provides the force needed to move a charge from one point to another, and is created by the separation of charges. The ease of this flow from one point to another is called conductance. Ohm's law states that:

$$I = gE$$

Where the current (I) is equal to the product of the conductance (g) and potential difference (E). This equation can also be put in terms of resistance (R), which is 1/g, or the inverse of conductance:

$$I = E/R$$

The total membrane conductance is a measure the ease of ion flow through all open ion channels in parallel in the cell membrane (Hille, 2001).

Resting Potential

Under resting conditions, all cells possess a potential difference across their plasma membranes. The inside of the cell is negatively charged compared to its environment**.** The resting potential can vary from -5 to -100 mV depending on the type of cell (Widmaier et al., 2009). For most amphibian oocytes, the resting potential is -50 to -60 mV (Slish, n.d.). A resting potential exists because there are more negatively charged particles inside the cell and more positively charged particles outside the cell. These positive and negative charges are attracted to one another. This creates a buildup of negative charges along the inner surface of the cell membrane, while the bulk of the cell contents remain mainly neutral. The magnitude of the resting potential depends on

the differences between the intracellular and extracellular ion concentrations, and variations in membrane permeability to individual ions.

The Nernst equation describes the equilibrium potential, for any ion:

$$E_{ion} = (61/z) \log (C_o/C_i)$$

The equilibrium potential can predict the direction of ion flow based on the intracellular and extracellular ion concentrations (Widmaier et al., 2009). According to the Nernst equation, potassium moves down its concentration gradient from a higher concentration inside the cell to a lower concentration outside the cell. This potassium-ion leak establishes a more negative intracellular region compared to outside. The movement of ions through channels establishes a transmembrane potential, which can then be recorded using Ohm's law, I = gE (Hartzell, 2008). Potassium ions leaving the cell, while sodium and chloride ions enter the cell help to create the negative resting potential of the cell membrane (Widmaier et al., 2009).

Acetylcholine (ACh) Receptors

Cell membranes not only contain ion channels, but they also expose receptors that activate specific channels. Arellano et al. (1998) found that *Xenopus laevis* oocyte membranes contain both cholinergic and purinergic receptors. Purinergic receptors respond to ATP and activate fast inward currents in the follicle cells, which surround the cell (Arellano et al. 1998). For this study we aimed to activate the cholinergic receptors with the addition of acetylcholine (ACh). Addition of ACh evokes an electrical response across the oocyte membrane and those of the follicular cells or both. Follicle cells are believed to release a maturation-promoting factor (MPF), which is responsible for oocyte

maturation (Longo, 1997). The follicle cells maintain a metabolic and electrical coupling with the oocyte via gap-junction channels (Arellano et al. 1998). These junctions allow the recording of the follicular currents to be taken by the insertion of electrodes in the oocyte (Rogelio et al., 1998). This was one reason why we chose to leave the follicle cells intact and manually separate the oocytes (see METHODS). On the other hand, Miledi and Woodward (1989) argue that the oscillatory chloride responses evoked by ACh are produced just in the oocyte itself.

Acetylcholine receptors (or cholinergic receptors) are classified as either muscarinic or nicotinic owing to their sensitivity to these drugs. The receptors in oocytes are muscarinic (Kusano et al., 1982). Muscarinic receptors operate through second messengers to activate ion channels. For example, they couple with G proteins to open potassium channels and also allow for the release of calcium via a G-protein cascade (Widmaier et al., 2009).

Xenopus laevis

Xenopus laevis oocytes are one of the most commonly used kinds of cell studied electrophysiologically. They are approximately 1mm in diameter, which makes them easy to handle. Oocytes are precursors to mature egg cells. They are classified into stages I-VI depending on their meiotic stage. Stages IV through VI oocytes are generally used in electrophysiology studies and are naturally arrested at the end of G2 phase of the meiotic cell cycle (Bernardo and Iverson, 1992). There are two poles present on oocytes, the animal pole, which is dark brown in color, and the vegetal pole, which is yellow. The vegetal pole mainly consists of the yolk, which provides its yellowish color.

Calcium Dependent Chloride Channels (CaCCs)

When ACh is added to the bath of the oocyte, muscarinic receptors on the oocyte membrane and follicle cells, or both, are activated (Arellano et al., 1998). This activates a phosphoinositidase enzyme, which produces a G-protein cascade and produces inositol 1, 4, 5- triphosphate (IP_3). IP_3 is a second messenger that stimulates the release of calcium (Ca^{2+}) from intracellular stores in the endoplasmic reticulum of the cell (Miledi and Parker, 1989; Slish, n.d.). Ca^{2+} may also enter the cell from the extracellular space (Machaca, 2006). The increase of Ca^{2+} within the cytosol activates Ca^{2+}-dependent chloride (Cl^-) channels along the plasma membrane of the oocyte. Chloride ions can then either enter or leave the cell.

The direction of the Cl^- through calcium-dependent chloride channels depends on three factors: membrane potential, Cl^- concentration gradient, and intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$). The build up $[Ca^{2+}]_i$ produces a depolarization of the cell membrane, which causes the membrane potential to become more positive. The depolarization in most cases is followed by a hyperpolarization of the cell membrane, which causes the membrane potential to become more negative. This may be produced by the Cl^- ions entering the cell and the leakage of K^+ ions out of the cell (Hartzell et al. 2005). This depolarization and repolarization creates an observable oscillation of the cell membrane potential. According to Kleckner (2008), this oscillation is possibly produced by the pulsating nature of Ca^{2+} being released from intracellular stores. The response gets smaller over time. This may be due to the desensitization of the receptor and ion channel, or the over-stimulation of the cell and its eventual death (personal communication N. Kleckner, November 12, 2008).

METHODS

Surgical Removal of Oocytes

Female *Xenopus laevis* frogs were obtained from a commercial supplier, *Xenopus Express Inc*. and stored on site by immersion in room temperature well water. The frogs were fed twice per week and kept in almost constant darkness in order to keep the oocytes from maturing. The mature frog used for surgery was chosen based on date of last operation, but the frogs were not operated on more than three times.

The chosen frog was first anesthetized using 0.2% MS-222 (ethyl 3-aminobenzoate methanesulfonate salt). The frog is fully anesthetized when it no longer tries to flip over when placed on its dorsal side. The frog is then placed ventral side up on a sterile surgical tray. A small, approximately 1 cm wide, incision through the skin and muscle layer on one side of the abdomen is made using a sterile scalpel and scissors as needed. Sterile scissors and forceps are used to remove the ovarian tissue and place into a petri dish containing Barth's solution. The Barth's solution consists of: 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 20 mM HEPES, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$. The pH was adjusted to approximately 7.4 with NaOH to approximate the frog blood pH (Kaplan and Payne, 1968).

Finally, the muscle wall and then the skin were sutured with one stitch in each. It was important that the skin and muscle not be sewn together, which allowed survival of the frog for use in future operations. The frog was then allowed to recuperate in a bucket of well water and separated from other frogs until recovered. Surgeries were staggered

so that the most recently operated frog was allowed to rest for 2-3 months before a second surgery was performed.

Preparation of Oocytes

After the oocytes were isolated from the ovary, they were separated using heavy gauge needles (see Slish, n.d.). This left the follicle cells intact around the oocytes. The oocytes are held together with connective tissue and blood vessels, which need to be broken in order to separate the oocytes individually. Cells were chosen in the IV to VI stage of development. Approximately 30-50 oocytes were gathered at a time and placed into a petri dish containing Barth's solution. These dishes were stored in a refrigerator at 18 ºC and could be kept healthy for approximately 4-6 days, depending on the frog.

IACUC approved protocol #A96-12-01, "Characteristics of Ion Channels of Drosophila's Cardiac Pacemaker." I received IACUC training from my advisor, Harold Dowse, before I began lab work in the summer of 2008.

Electrophysiological Recordings

Glass micropipette electrodes were prepared using an electrode puller. This device applies constant tension and heat on a glass capillary tube. As the glass becomes soft, the puller arm stretches the weakened area until it eventually breaks and a very fine point is produced on the capillary tube (Slish, n.d.). Two micropipette electrodes can be made from one glass capillary tube. The tip was broken in case the end was closed off, but this was carefully done to prevent the end from becoming too blunt.

The electrodes were filled with 3M KCl and mounted onto plastic pipette holders containing silver electrode wires. These silver wires were coated with silver chloride by immersing them in hypochlorous acid, Clorox® bleach. For the Warner model OC-725C oocyte clamp apparatus, two electrodes, one a voltage electrode and the other a current electrode, were prepared and mounted to the oocyte clamp. The experiments were conducted using pClamp software and Warner amplifier.

Recording equipment

Two procedures were used for electrophysiology recordings. The following protocol is for the whole-cell clamp approach for the model OC-725C oocyte clamp. The system was first set to the settings as follows in Table 1.

Table 1. Pre-recording settings of the model OC-725C oocyte clamp (Orono, ME 2008).

Section	POWER	OFF
Bath Electrode	1 MONITOR OUTPUT	1 V/µA
	GAIN SELECT	x1
Commands	HOLD	0mV
	+/OFF/-	OFF
Clamp	MODE SELECT	OFF
	CLAMP GAIN	OFF (fully ccw)
	CLAMP DC GAIN	OUT

Step-by-Step Protocol: Warner

The power should be turned on and the setup checked for the above settings (Table 1). Advance electrodes into the bath and make sure the voltage is displayed. If no voltage is displayed, there may be a bubble in the pipette. Use the OFFSET knobs to

adjust the potential and current to 0mV. If the potential will not stabilize at 0 mV, the voltage holder may be faulty. Next, test resistance by dividing potential by 10 nA (move decimal to left by one). The resistance of the electrodes should be less than 4 MΩ. Finally, slowly advance the electrodes into the oocyte membrane.

Step-by-Step Protocol: Dagan

The following protocol is for the Dagan Amplifier used for BIOPAC experiments. The bath should contain 4 mL of Barth's solution. The bath is connected to the differential using an agar bridge to eliminate most resistance in the system. Turn amplifier, computer, and oscilloscope on. Advance the electrode into the bath and zero the electrode in bath using the junction knob. Next, check electrode resistance by first turning the Series Resistance on and Test Pulse to HIGH. Turn the Series Resistance dial until the peaks observed on the oscilloscope are eliminated and a flat line is observed. Then, check current holding potential to make sure no current is being sent through the system. Re-zero the resting potential (V_{rest}) until a constant 0 mV is observed using the junction knob. Finally, insert the recording electrode into the oocyte membrane. Once V_{rest} is stabilized turn computer and BIOPAC (BSL Pro 3.7) on, and click start to begin recording. (Note: V_{rest} for a healthy oocyte should be no less than -15 mV).

Preparation of ACh

Acetylcholine chloride (Sigma Company) was stored in a tightly closed container, in a cool, dry area. It is stable under ordinary conditions of use and storage, but heat, flames, ignition sources and incompatibles must be avoided for safety precautions. For

the Dagan system, a dilution series was carried out to make five concentrations of ACh: 1 M, 100 mM, 10 mM, 1 mM, 100 µM.

For the Warner system, 10 drops of 0.1 µM of ACh were added directly on the oocyte. For the Dagan setup, 1 mL of ACh solution was added to the bath after approximately 600 seconds of stabilization of the cell resting potential. Adding the concentrations to the bath containing 4 mL of Barth's solution diluted the concentrations to 100 mM, 10 mM, 1 mM, 100 µM, and 10 µM respectfully.

RESULTS

The application of acetylcholine induced notable oscillations in healthy *Xenopus* oocytes. The separation process we used to pull apart the cells caused low survivability. A majority of the oocytes were disfigured in some way during the separation process so that it was impossible to record from them. Only about 50-70% of the oocytes could be used from the original harvest from the frog. Of the approximately 30-50 oocytes that survived this procedure, fewer than 20 oocytes were healthy enough to be poked and recorded. A majority of these unhealthy oocytes exhibited a resting potential more positive than -10 mV. Also, several of the oocytes were too soft to be poked with a micropipette electrode.

Oocyte Ion Channel Oscillations

Warner Recording System

The majority of *Xenopus laevis* oocytes exhibited notable ion channel oscillations induced by application of 0.1 µM concentration of acetylcholine. Several oocytes produced oscillations in a rhythmic manner (table 2). Out of the 20 oocytes, approximately nine were considered arrhythmic and not shown in table 2. This rhythmicity was determined by the use of autocorrelogram and Maximum Entropy Spectral Analysis (MESA). For the rhythmic oocytes, the average oscillation period was approximately 30 seconds to 110 seconds.

Table 2. Period of oscillations recorded using the Warner recording system and 0.1 µM ACh. The oscillation mean was 71.2 seconds with a standard deviation of 42.0 seconds.

Oocyte	S2	S3	S4	S5	S6	S7	S8	S11	S12	S17	S18
Period (sec)	47.8	63.7	43.70	163.1	39.1	100.2	122	72.0	70.2	39.5	22.6

The first set of oocyte ion channel oscillations was recorded using the Warner amplifier system, model OC-725C oocyte clamp. Ten drops (from a plastic disposable pipette dispenser) of 0.1 µM ACh were added to the oocyte bath. The oscillations varied in rhythmicity, but notable membrane potential oscillations were observed and recorded for approximately 8 minutes (Figure 1). Note that the amplitude is an arbitrary number produced by the computer program and is just used to show the oscillation, not its magnitude.

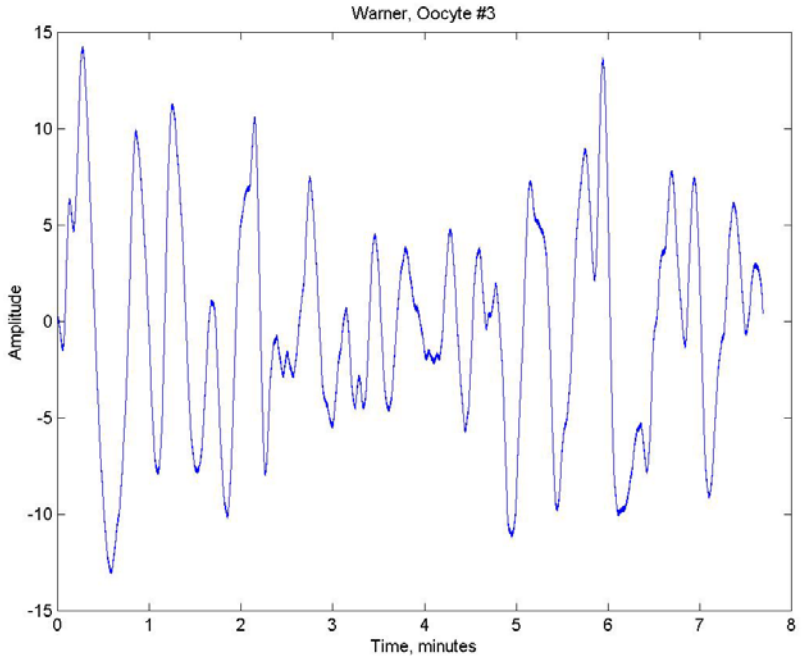

Figure 1. Warner amplifier oscillations of 'Oocyte 3' produced with 0.1 µM ACh. August 5, 2008. Orono, ME.

To confirm the presence and significance of any rhythmicity, an autocorrelogram was created (Figure 2). An autocorrelogram is a statistical analysis that computes a rhythmicity index in order to determine the regularity of the rhythm. Frequency and period may be measured using the data from this plot (Dowse, 2009). The horizontal lines represent the +/- 95% confidence interval, or significance of a peak. Any wave equaling or exceeding this interval provides reason to believe the cell is producing a significant periodic oscillation.

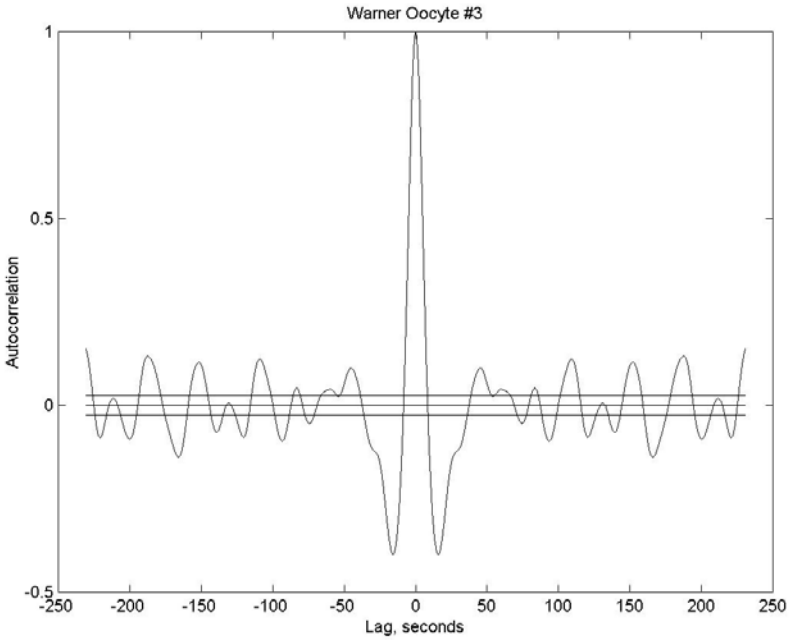

Figure 2. Autocorrelogram of 'Oocyte 3' oscillations, with +/- 95% confidence interval produced with 0.1 µM ACh. August 5, 2008. Orono, ME.

MESA is another tool used to show the rhythmicity of the oscillation peaks. There is a strong correlation seen between the sharpness of the peaks and the regularity of the rhythms (Dowse, 2009). The highest peak can be used to determine the period of the oscillations (table 2; Figure 3).

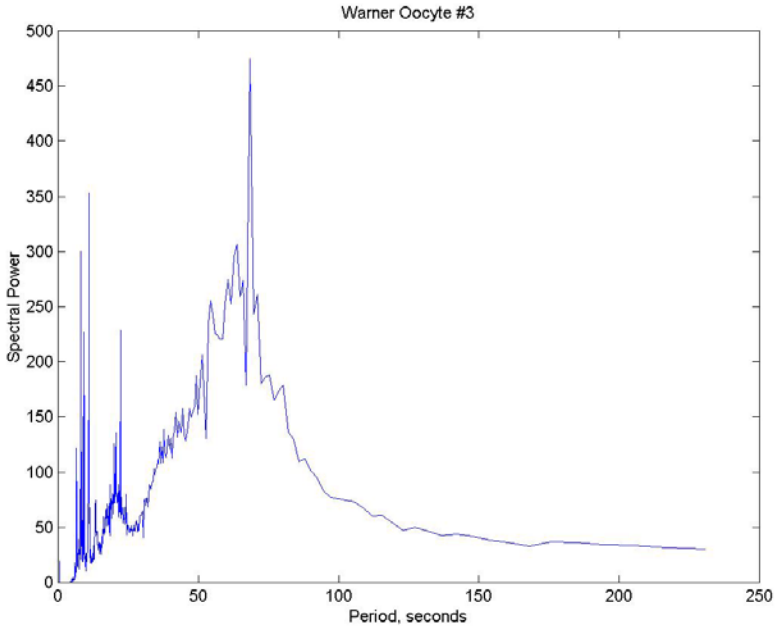

Figure 3. MESA of 'Oocyte 3' oscillations produced with 0.1 µM ACh. August 5, 2008. Orono, ME.

Figures 4-9 provide additional examples of ion channel oscillations and statistical analysis of the rhythmicity.

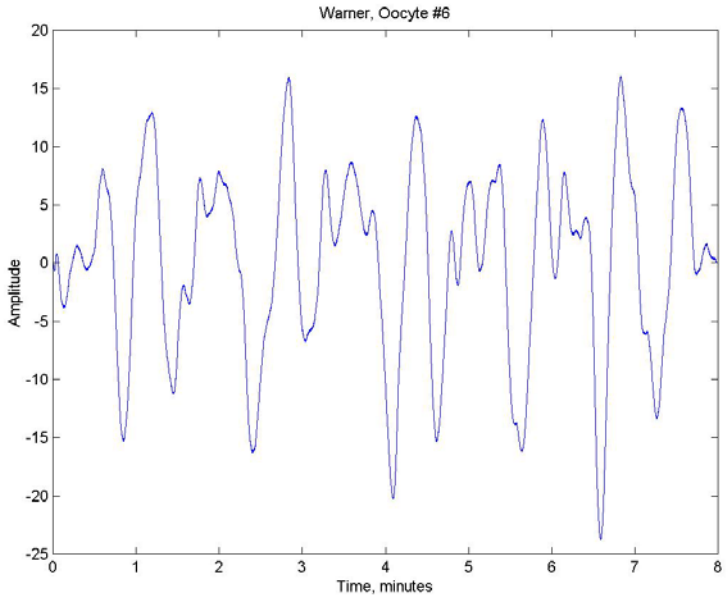

Figure 4. Warner amplifier oscillations of 'Oocyte 6' produced with 0.1 µM ACh. August 19, 2008. Orono, ME.

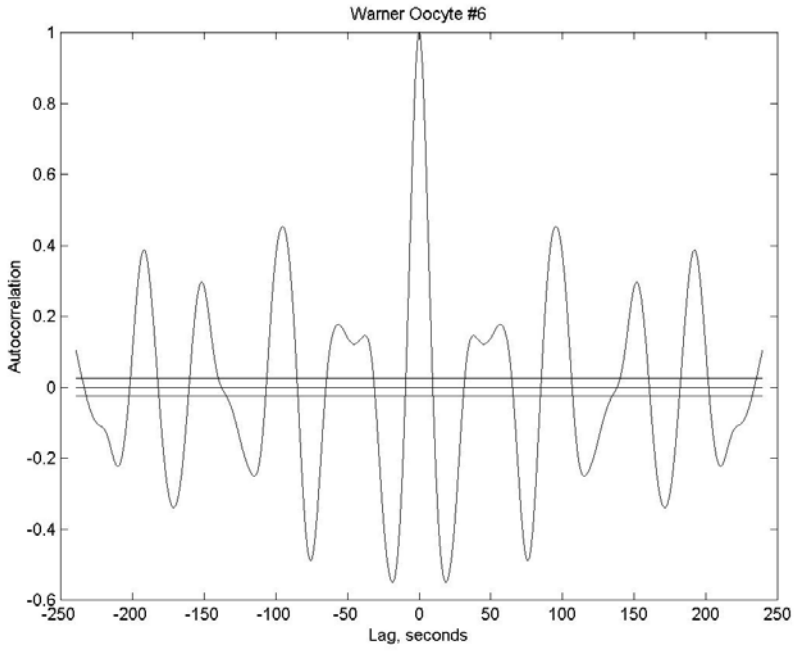

Figure 5. Autocorrelogram of 'Oocyte 6' oscillations, with +/- 95% confidence interval produced with 0.1 µM ACh. August 19, 2008. Orono, ME.

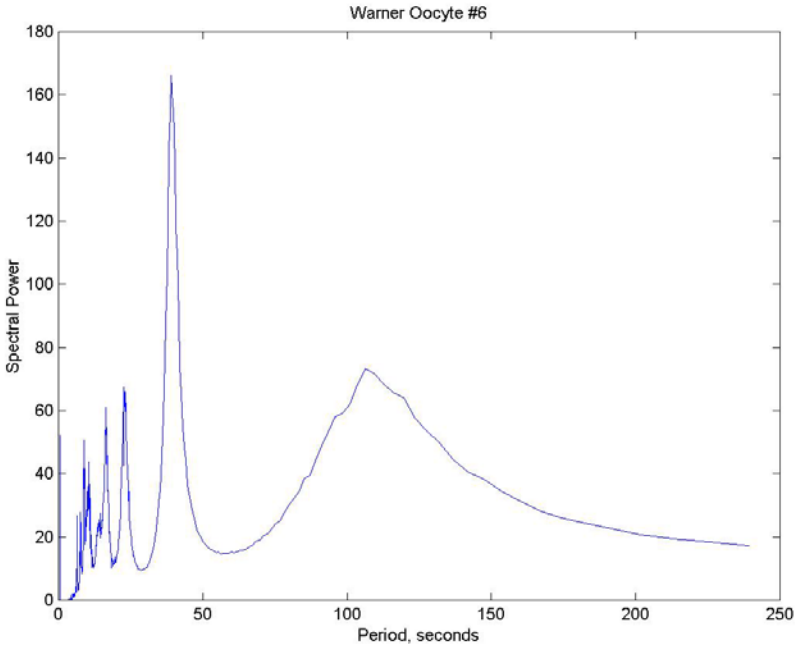

Figure 6. MESA of 'Oocyte 6' oscillations produced with 0.1 µM ACh. August 19, 2008. Orono, ME.

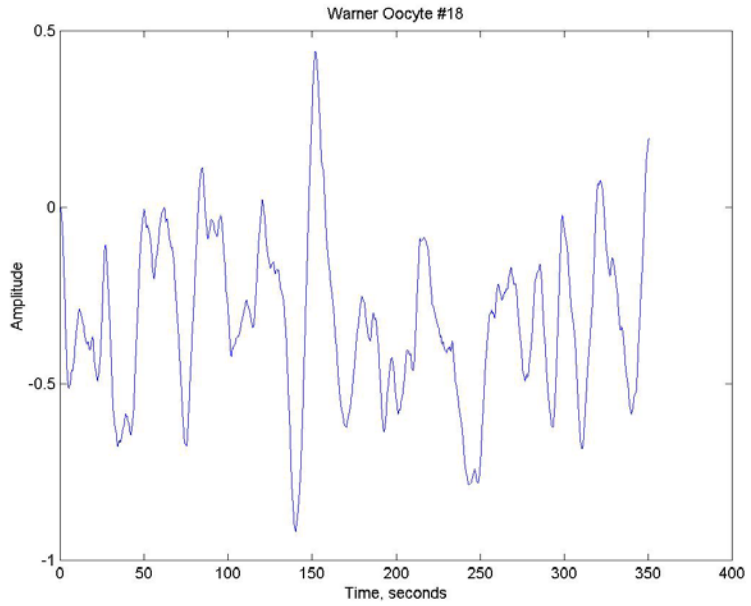

Figure 7. Warner amplifier oscillations of 'Oocyte 18' produced with 0.1 µM ACh. October 30, 2008. Orono, ME.

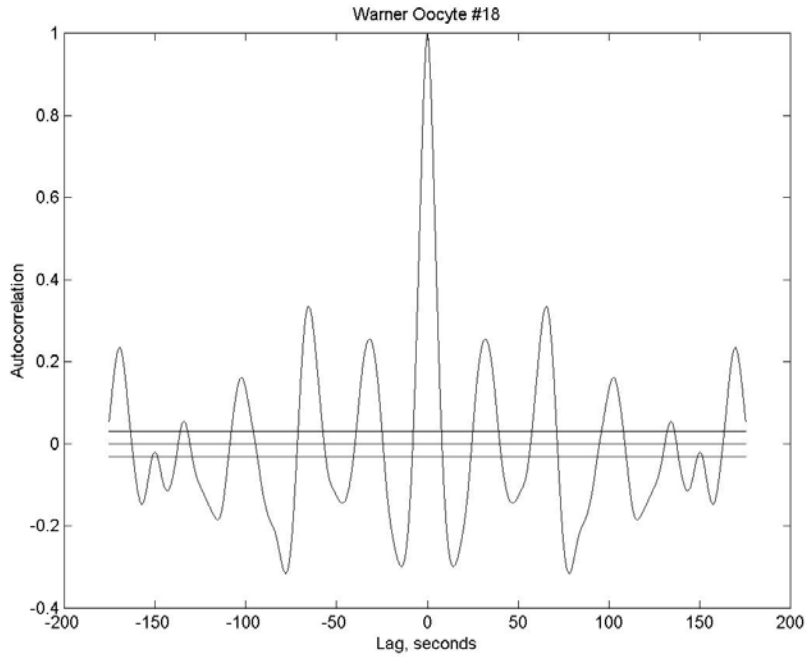

Figure 8. Autocorrelogram of 'Oocyte 18' oscillations, with +/- 95% confidence interval produced with 0.1 µM ACh. October 30, 2008. Orono, ME.

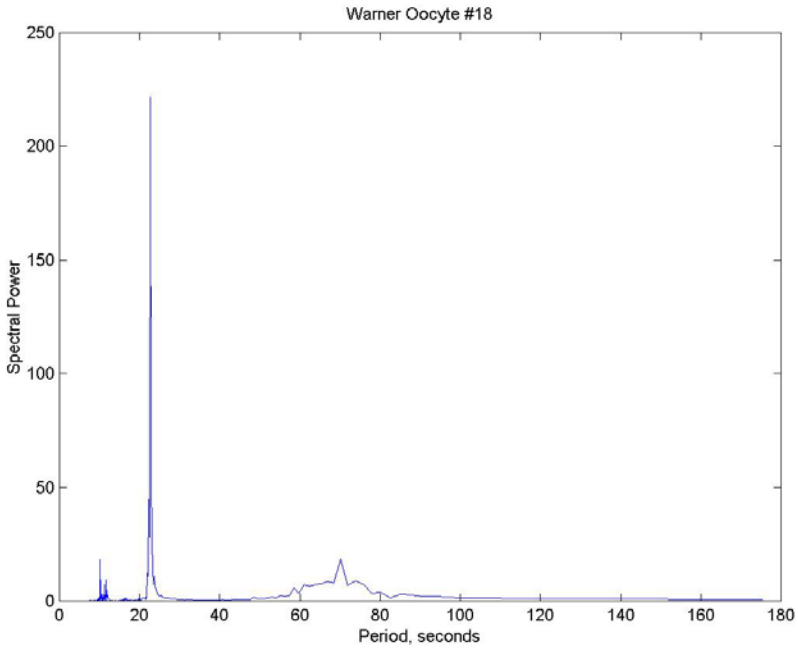

Figure 9. MESA of ‘Oocyte 18’ oscillations produced with 0.1 µM ACh. October 30, 2008. Orono, ME.

Dagan Recording System

Ion channel oscillations were also shown using the Dagan recording system. This system was used to prevent much of the noise that was found with the Warner system. However, more notable ACh ion channel oscillations were found using the Warner recording system. Note that the artifact at approximately 10 minutes is a 1 mV calibration. This indicates that the oscillations are less than 1 mV change in voltage (Figure 10).

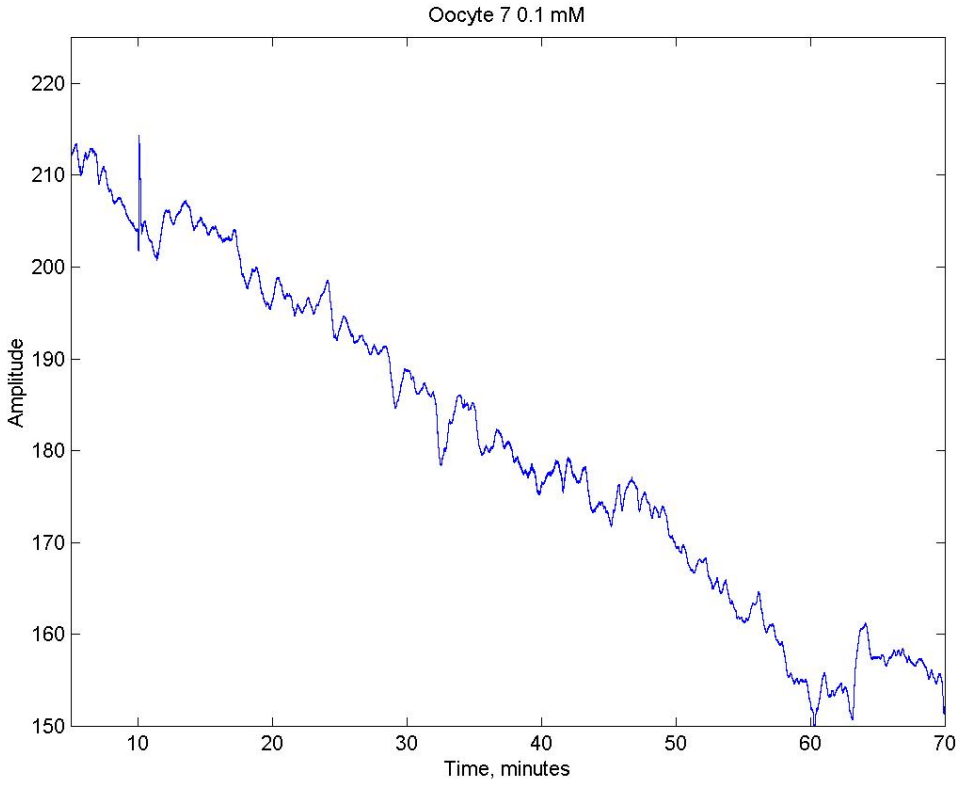

Figure 10. Dagan oocyte oscillations produced with 0.1 µM ACh. January 7, 2009. Orono, ME. The amplitude is measured in mV and should be multiplied by -0.1 mV to determine the approximate membrane potential.

The ACh dilution series accompanied several sources of error, such as timing of ACh addition (see DISCUSSION). For those data obtained without significant error, showed significant membrane-potential oscillations. For 1 mM and 100 mM a notable oscillation and depolarization could be observed (Figure 11, Figure 12). The downward slope indicates the depolarization (membrane potential is getting more positive) of the oocyte membrane.

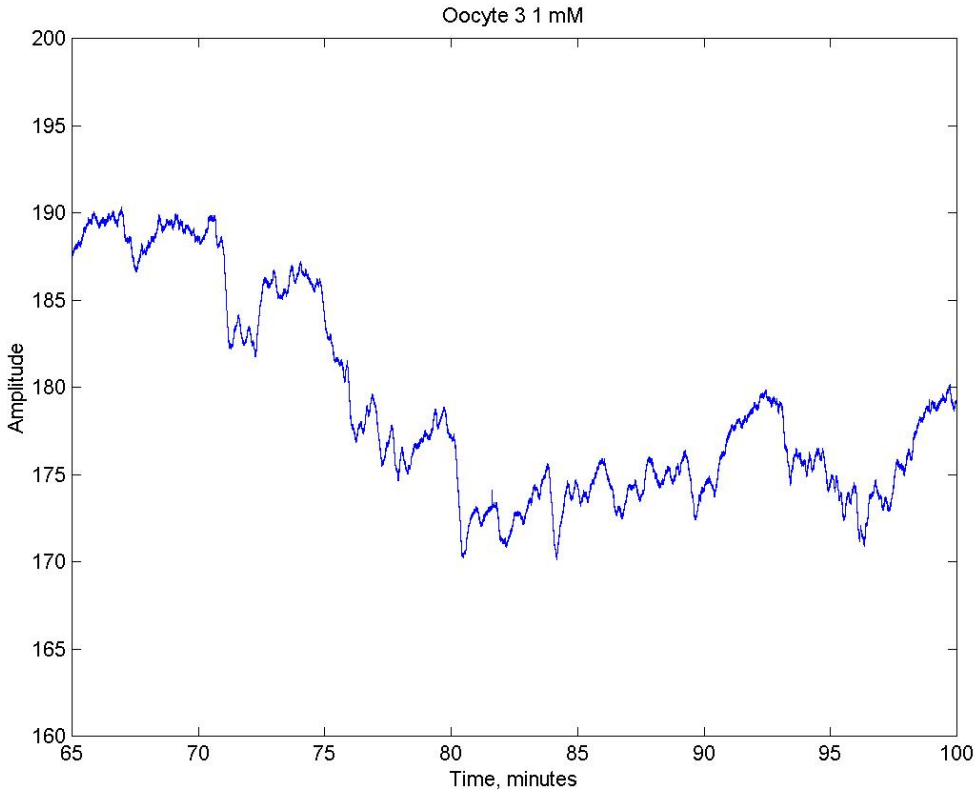

Figure 11. Dagan oocyte oscillations produced with 1 mM ACh. February 27, 2009. Orono, ME. The amplitude is measured in mV and should be multiplied by -0.1 mV to determine the approximate membrane potential.

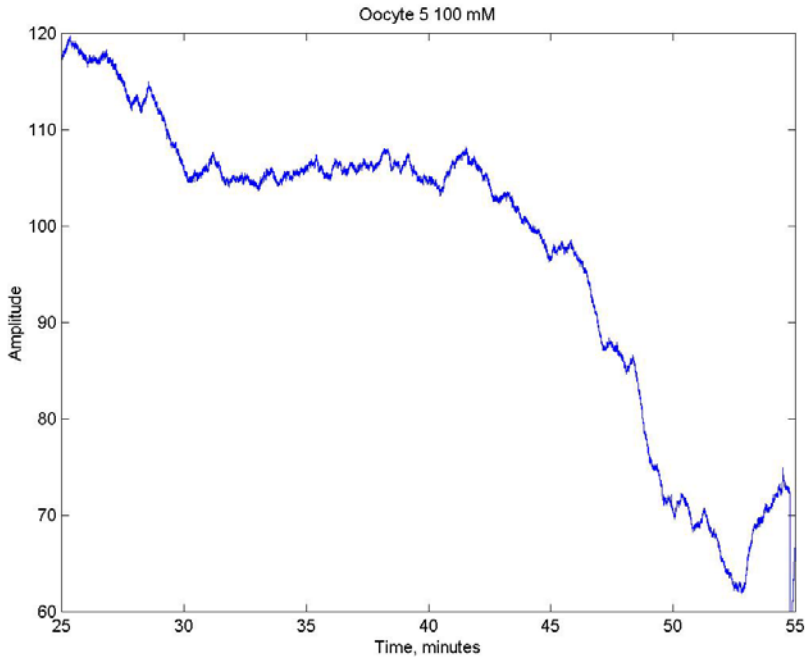

Figure 12. Dagan oocyte oscillations produced with 100 mM ACh. February 27, 2009. Orono, ME. The amplitude is measured in mV and should be multiplied by -0.1 mV to determine the approximate membrane potential.

The data gathered from Dagan amplifier did show oscillations, but not as significant as the oscillations observed from the Warner system. However, BIOPAC was useful in gathering information about the ion channel itself. As noted in the introduction, calcium dependent chloride channels are known to produce a depolarizing effect on the cell membrane (Figure 13 and 14). These studies also show that a majority of the oocytes depolarized overtime due to cell death (Figure 13).

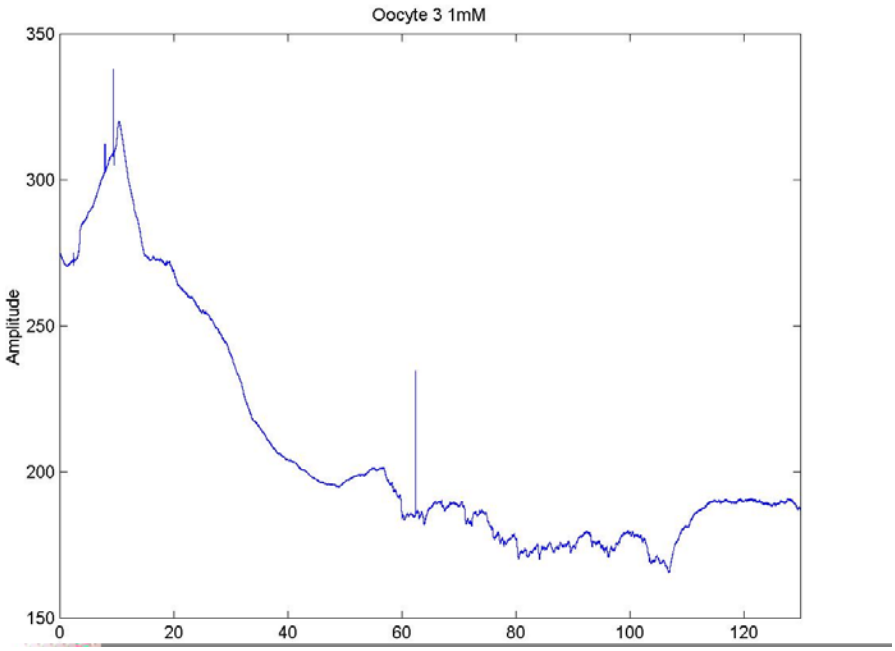

Figure 13. Dagan oocyte ion channel activated with 100 mM ACh. February 27, 2009. Orono, ME. The amplitude is measured in mV and should be multiplied by -0.1 mV to determine the approximate membrane potential. (*Note: X-axis in Time, minutes).

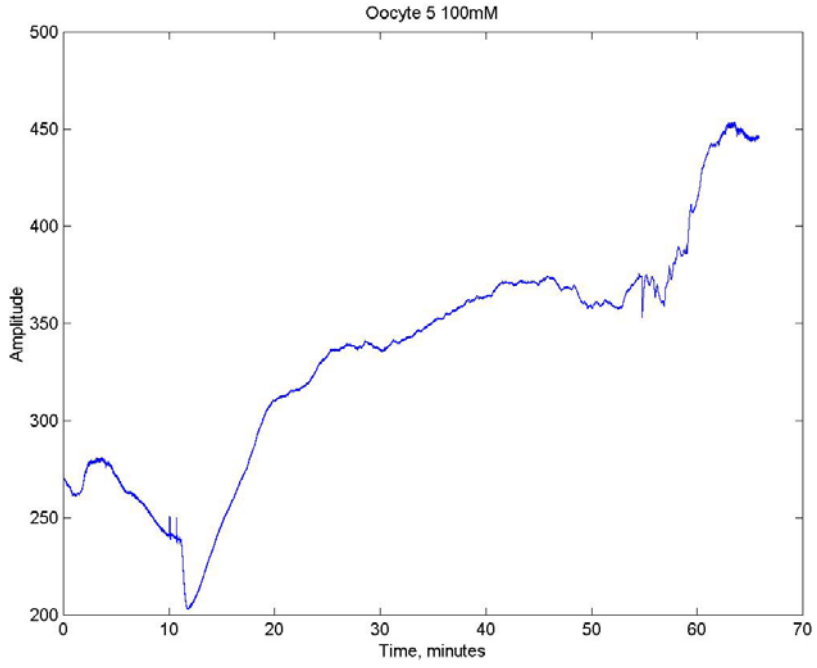

Figure 14. Dagan oocyte ion channel activated with 100 mM ACh. February 27, 2009. Orono, ME. The amplitude is measured in mV and should be multiplied by -0.1 mV to determine the approximate membrane potential.

DISCUSSION

Survival of Oocytes

The quality of the oocytes varied among frogs. The best indicators of oocyte health were the pigmentation of the animal pole, number of underdeveloped oocytes present, and the ease of electrode penetration through the oocyte membrane. A uniform dark brown color on the oocyte indicated a healthy oocyte. This could be determined as soon as the eggs were separated. It could also be observed that the lower number of underdeveloped oocytes present, the healthier the mature oocytes. Healthy oocytes tended to resist penetration and exhibited resting potentials of -30mV or more negative. However, the majority exhibited resting potentials more positive than -15mV. This made it difficult to obtain reliable data. New frogs tended to have the healthiest oocytes and more negative resting potentials, than the older frogs used.

Ionic Currents Recorded from Oocytes

Berridge (1988) found that a low concentration of acetylcholine ($1X10^{-7}$ M) induced regular oscillations in the cell membrane potential. He also discovered that the oscillatory frequency increased as the concentration of acetylcholine was increased. We also found that this rather low concentration (0.1 μM) of ACh could induce such membrane potential oscillations. However, our data were not extensive enough to determine the effect of ACh concentration on membrane potential oscillations.

Several things may have caused these oscillations. The most plausible explanation is the pulsatile nature of Ca^{2+} being released from intracellular storage by the

second messenger IP_3, which is activated by ACh. This release of Ca^2 may have played a role in depolarizing the cell membrane. The Ca^2 then opened Ca^2 dependent Cl^- channels, which allowed for Cl^- ions to enter the cell, producing a hyperpolarization of the cell membrane (Miledi and Parker, 1989; Kusano et al. 1982).

Conclusions

Several other studies have described membrane potential oscillation produced by acetylcholine (Arellano et al., 1993, 1998; Berridge, 1988; Dascal et al., 1985; Kusano et al. 1982; Miledi and Parker, 1989; Slish, n.d.). However, none of these studies have shown if there is any regular rhythmicity to the oscillation. Our analyses show that the period is approximately half a minute to a minute and a half in length. Ultraviolet radiation has the potential to change the oscillation, making it arrhythmic, or damage cell functions.

Future studies

For future studies it will be important to take into account the variability of health among oocytes. Frog oocytes are one of the easiest ways to record specific ion channels. It is important to remember that when injecting an RNA sequence to create a particular ion channel transport protein, the oocyte already possesses its own membrane channels. These channels could also be recorded when this sequence is injected and used for membrane recordings. For example, the oocyte contains muscarinic ACh receptors that activate native calcium-dependent chloride channels by releasing calcium from

intracellular stores. The oocyte also contains potassium channels that help to establish the resting potential of the cell membrane.

Kusano et al. (1982) discovered that ACh depolarizes the membrane in a dose-dependent manner. The lowest concentration used was $1x10^{-9}$ M. The most consistent results we recorded used $1x10^{-7}$ M ACh. For future studies it will be interesting to pay more attention to differences in the periods of the oscillations produced with different ACh concentrations. Most other studies on oocytes use a bath that has Barth's solution constantly perfused. This would keep the bath clean and perhaps allow the recordings to be more consistent between the oocytes. It may have also allowed for a better recording, or more negative resting potential.

It would also be interesting to find a way to control the leakage of KCl from the electrode. This leak may have played a role in determining the Cl^- ion equilibrium potential (E_{Cl}) and the way in which the chloride ions traveled across the membrane. It also may have produced different results depending on the micropipette electrode used and the size of the opening of the broken tip. More careful observations of the starting and ending membrane potentials will also be important to note in future studies. Additionally, it may help to be more careful when to dispense the ACh, letting the potential of the membrane stabilize before the addition of the drug. Overall the procedure we have created and data analyses used will be a great protocol for future experiments, especially those examining the effects of ultraviolet radiation.

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