The Effects of Ethanol on Cell Signaling Demonstrated by Change in Heart Rate of Mercenaria mercenaria

Jeremy Beech
Wheaton College
Norton MA, 02766

Introduction:

The purpose of this experiment is to look at the effects of ethanol on cell signaling. It is known that ethanol (C2H60) is a small soluble molecule that, in low concentrations, can increase the fluidity of cell membranes (Cooper,2004). Since one way of cell signaling is through the binding of signaling molecules to clustered receptors on the plasma membrane of a cell, an increased fluidity of the plasma membrane could result in a dispersion of receptors around the cell and slow cell signaling. There is also the possibility that ethanol directly affects signaling molecules by either interfering with their trafficking or effecting the activity of intracellular signaling cascades (Morrow, 2004). To test the effects of ethanol on cell signaling a specific cell signaling pathway must be chosen to study.

Epinephrine is a small molecule that can act as either a neurotransmitter or a hormone to trigger the breakdown of glycogen to glucose in muscle cells in preparation for muscle contraction (Cooper, 2004). Epinephrine is one of the key molecules in stimulating the fight-or-flight response. This then results in the elevation of heart rate and other response within the organism (Wikepedia). Epinephrine directly affects heart rate (Cooper, 2004) making it an ideal chemical to use to visualize cell signaling because heart rate is something that can be measured as quantitative data.

Live steaming clams (*Mercenaria mercenaria*) were used in this experiment because they were readily available and their tissues are easily viewed under a dissecting microscope. The heart rate of clams in the closed and resting state is approximately 2 beats per minute but can raise to 20 beats per minute during extreme nervous excitement (thestraightdope). In this experiment I will be comparing data of the heart rate of *Mercenaria* in solutions of ethanol and epinephrine to see their effects on cell signaling.

Since epinephrine is a stimulant it will increase the heart rate of Mercenaria when added to the seawater buffer, however addition of ethanol will slow the heart rate when added to the seawater buffer. In the presence of both epinephrine and ethanol the heart rate will decrease because of the effect ethanol, either directly on specific components of the signaling pathway or indirectly by dispersing the receptors because of an increased fluidity of the plasma membrane.

Materials

Ethanol
Epinephrine 183.2 FW
30 clams (Mercenaria mercenaria)
dissecting dish
dissecting microscope
probe
scalpel
sea water
thermometer
pipet
pipetman

Methods:

Dissection of Clams

Start by cutting the adductor muscle as close to the shell as possible starting from the umbo and continuing around the shell using a scalpel. Avoid damaging any of the other tissues within. Once the adductors had been cut, the shell opened. The top shell, free from the clam, was pulled back and off. The dissected clam was then placed in 200mL of filtered sea water (kovacs).

Find Clam Heart Rate

1. Put clam in dissecting dish with 200mL filtered sea water at room temperature (27 degrees C).
2. Put clam underneath dissecting microscope.
3. Pull back tissue covering gills and heart with probe and tweezers.
4. Record movie of heart rate for interval of one minute.
5. Count heart rate over interval of one minute.
6. Repeat step in Dissection of Clams and steps 1-8 of Find Clam Heart Rate for two more clams.
7. Record calculations and any observations.

(Kovacs)

Prepare Epinephrine Solution

Want to use concentration of 5x10^-5 M on cells
= .05mM
= 50mM

epinephrine is 183.2 FW
.183 g/L = 1mM
183 mg/L = 1mM
1.83 mg/L = 10mM
9.2 mg/L = 50mM

make 9.2 mg/10ml = 100 x strength for stock solution.

Measured 13mg epinephrine

9.2 / 10 = 13 / X = 13 / 14ml
stock solution is 13mg epinephrine in 14ml distilled H20
(R.L Morris)

Clam Heart Rate Under Influence of Ethanol

1. Dissect a clam by following steps for Dissection of Clam.
2. Keep clam in 200mL filtered seawater.
3. Find heart of clam by following directions for Find Clam Heart Rate.
4. Record video of heart rate for one minute.

5. Add 4mL of ethanol to the 200ml of filtered seawater with clam to create a 2% v/v solution of ethanol.
6. After 1 minute, take video of heart rate for one minute.
7. Count heart rate over interval of one minute.
8. Record calculations and any observations.
9. Three minutes after the ethanol was added to the filtered seawater solution, record another movie of the clam heart rate.
10. Record heart rate and any observations.
11. Repeat steps 1-10 with two more clams.

(Kovacs)

Clam Heart Rate Under Effect of Epinephrine

12. Dissect a clam by following steps for Dissection of Clam.
13. Keep clam in 200mL filtered seawater and find heart rate following steps for Find Clam Heart Rate.
14. Record video of heart rate for interval of one minute and record heart rate.
15. To create a 5 x 10^-5 solution of epinephrine in seawater, add 100µM of stock solution epinephrine to the 200ml of filtered sea water with clam.
16. After 1 minute, take video of heart rate for one minute.
17. Count heart rate over interval of one minute.
18. Record calculations and any observations.
19. Three minutes after the epinephrine was added to the filtered sea water solution, record another video of the clam heart rate.
20. Count heart rate over interval of one minute.
21. Repeat steps 12-20 with two more clams and record results.

(Kovacs)

Procedure II

A second procedure was run to account for the effect of stress on the clams heart rate during opening. In this procedure three clams were dissected, one in each of the three solutions. Instead of recording a movie and heart rate first in the buffer solution, the clams were added straight to their testing solutions.

Clam Heart Rate in Buffer

1. Dissect a clam by following steps for Dissection of Clam.
2. Find heart of clam by following steps for Find Clam Heart Rate.
3. Record a 1 minute movie of clam heart rate after opening, after 2 minutes, and after 5 minutes have elapsed.

Clam Heart Rate in Strong Epinephrine Solution

4. Add 300 µM of epinephrine to 200ml filtered sea water solution with clam in dissecting dish.
5. Repeat steps 1-2 in procedure II.
6. Record movie of clam heart rate after opening, after 2 minutes, and after 5 minutes.

Clam Heart Rate in Ethanol Solution

7. Add clam to 200ml or 2% v/v ethanol solution in dissecting dish
8. Repeat steps 1-2 in procedure II
9. record movie of clam heart rate after opening, after 2 minutes, and after 5 minutes.

Results:

Figure 1: clam heart relaxed

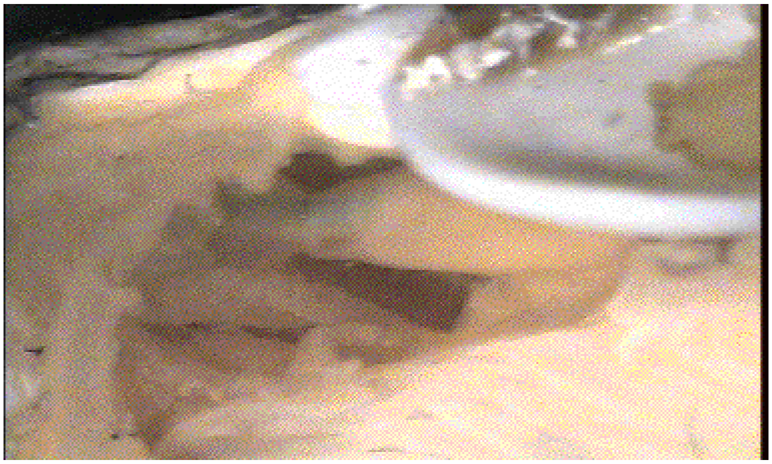

figure 2: clam heart contracted.

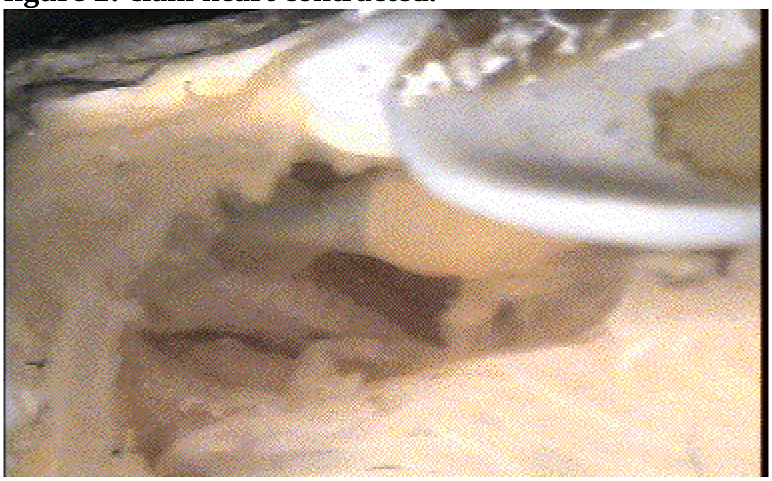

Table 1: Clam heart rate in 2% v/v ethanol.

	Average
Starting HR beats/min	15
HR after 3 min beats/min	15
HR after 6 min beats/min	12

Treatment of clams in 2% v/v ethanol showed a slight decrease in the average heart rate of clams over 3 minutes. Total decrease was 3 beats/min

Table 2: Clam heart rate in 5x10^-5 M epinephrine solution.

	Average
Starting HR beats/min	11
HR after 1 min beats/min	10
HR after 3 min beats/min	9

Treatment of clams in 5x10^-5 M epinephrine showed a steady decrease of heart rate over 3 minutes. Total decrease was 2 beats/min.

Table 3: Average change in heart rate.

	Average change
2% v/v ethanol	-2.6
5x10^-5 M epinephrine	-1.6

Treatment of clams in 2%v/v ethanol solution had a decrease in heart rate of 2.6 beats/min. Treatment of clams in 5x10^-5 M epinephrine had a decrease in heart rate of 1.6 beats/min

Table 4: Clam heart rate over time from opening (procedure II).

	HR after	HR after 2	HR after 5	Change in

	opening beats/min	min. beats/min	min. beats/min	HR
calm control	18	15	11	-7
clam in 2% v/v ethanol	15	12	9	-6
clam in 300µM epinephrin	15	16	15	0

Treatment of clams in 2%v/v ethanol solution and clams in a buffer of sea water had a decrease in heart rate. Treatment of a clam in 300µM **epinephrine had a constant heart rate.**

Figure 3: clam heart rate over time from opening.

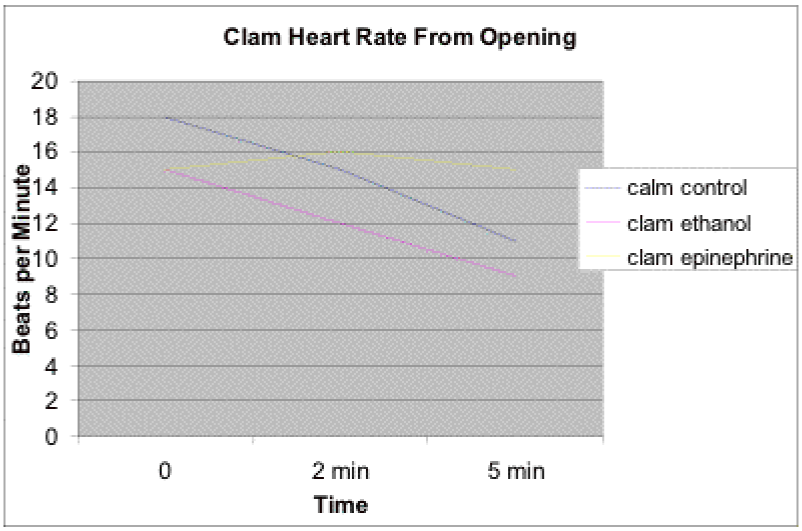

Figure 2: The heart rates of the clams in the control and clams in the ethanol solution showed a steady decrease over the five minutes. The heart rate of the clam in epinephrine stayed constant through the five minutes.

Discussion:

Based on my preliminary results I support my hypothesis that addition of epinephrine in a buffer of seawater results in an increased heart rate of Mercenaria mercenaria. Also my preliminary results suggest that Mercenaria mercenaria has a decrease in heart rate when placed in a solution of 2% ethanol and seawater.

Opening of the clam would be considered a stressful situation inducing the fight or flight response, previously mentioned, elevating the heart rate to between 15 and 20 beats per minute (thestraightdope). After a few minutes the heart rate began to decrease as shown in **figure 3**. This is most likely because the clam was dying. It was assumed that the rate of death was equal for all clams.

Ethanol is a depressant and has been used in the past as an anesthetic which might suggest that the heart rate of a clam would decrease once being opened in the same way that the clam in the buffer did (3rd1000, 1999). As can be seen in **figure 2** and table 3, the heart rate for clams in solution of ethanol did indeed decrease by an average of 2.6

beats/min over the time tested.

The clams tested in 5x10^-5 M (100µM) epinephrine solution had an average decrease in heart rate of 1.6 beats/min over the time tested. When compared to the average decrease in heart rate for clams treated in ethanol, this data suggest that epinephrine may prevent the rapid decrease in heart rate of a clam following opening. The heart rate for the clam tested in 300µM epinephrine for the most part stayed constant either at 15 or 16 beats per minute over the five minutes. This is what I expected since epinephrine stimulates the breaking down of glutamate to glucose for muscle contraction (Cooper, 2004).

In the first procedure it was hard to determine much difference between the heart rates for clams treated in solutions of ethanol from those treated in epinephrine and only the averages showed any significant differences. This probably has to do with the concentration used. Clams treated in a stronger concentration of epinephrine other then 5x10^-5 M (100µM) might have a more constant heart rate as observed when 300µM epinephrine was added. Also the time of exposure could be increased in the future to obtain more data. Because there was not enough of a difference in heart rates between ethanol and epinephrine at the concentrations used, I did not feel comfortable in continuing further experiments until I had conducted more tests and collected more evidence with different concentrations. Due to time constraints further testing was not possible and I was not able to test my hypothesis that a solution of ethanol and epinephrine applied to a clam at the same time would lower the clams heart rate.

The next experiment that should be run is a test of heart rate when a solution of epinephrine and ethanol are added at the same time to the clam. As seen when a stronger concentration of epinephrine was used (300µM), the heart rate remained constant. If ethanol does indeed interfere with epinephrine cell signaling of the heart then this is where it would be noticeable because the clam would be exposed to the effects of both drugs at the same time. I predict that if ethanol and epinephrine are added at the same time, the heart rate will decrease. According to Morrow's paper "Ethanol Effects on Cell Signaling Mechanisms", ethanol interferes with protein kinase A (Morrow, 2004). Protein kinase A is responsible for two target enzymes, Phosphorylase kinase, and the enzyme glycogen synthase in the metabolism of glycogen (Cooper, 2004). If protein kinase A is interfered with, it can not properly phosphorylate its targets preventing the metabolism of glycogen. My results suggest that this is what happens in clams when they are exposed to an ethanol solution and may be a factor in decreasing heart rate.

Works Cited:

Morrow, Leslie et al. 2004. "Ethanol Effects on Cell Signaling Mechanisms." Alcoholism: Clinical and Experimental Research, Vol 28, No 2: pp. 217-227

"Wikepedia." Wikimedia Foundation, Inc. November 2005. http://en.wikepedia.org/wiki

Cooper G.M., Hausman, R.E. 2004. The Cell: A Molecular Approach, 3rd Ed. ASM Press, Washington D.C

Personal correspondence with Esther Kovacs. November, 2005

Personal correspondence with R.L Morris. November, 2005

http://www.3rd1000.com/ethanol/ethanol.htm