

# [Voltage gated ion channels in daphnia heart](https://assignbuster.com/voltage-gated-ion-channels-in-daphnia-heart/)

It may be possible to use other tissues rather than that of higher animals as an educational tool for the study of pharmacological drugs. Previous experimental studies were carried out in order to determine how closely related the basic electrophysiology of daphnia heart was to the human heart. Work such as that of Krijgsman and Krijgsman – Berger (1950, 1951) indicated that the automatism of the heart of insects has a mechanism which is neurogenic. An alternative view, however, revealed that the hearts of daphnia are infact myogenic (Needham 1950). A close relation to the human heart would provide a useful model with which to study mechanism of cardioactive drugs. Since only a few drugs have been tested on the daphnia heart, it seemed useful to test other drugs to see whether the responses of the daphnia heart correlated with the responses of the drugs to the human heart. Carrying out these experiments would show how useful a daphnia heart would be for education purposes.

## Daphnia Heart

Stein and Richter carried out ultrastructural studies on the heart of the daphnia in order to determine the structure of it, specifically as to whether the heart was striated. They found that the heart wall was one cell thick (occasional regions were five cells in thickness), the cells were thin and contained many mitochondria, long striated myofibrils and an abundance of sacroplasm with an irregular indented cell surface.

From this research, it can be concluded that the structural features of the daphnia in many respects are quite similar to that of striated and cardiac muscle of other animal species, therefore daphnia could be used for educational purposes in undergraduate study. Since studies have not been carried out using cardioactive drugs, specifically channel blockers, we set out to experimentally determine how closely related the daphnia heart was to the mammalian heart and whether the daphnia could be used in undergraduate study.

The advantages of using a daphnia in experimental studies are the fact that it is transparent and one cell thick, therefore viewing the heart would be trouble-free. Using a daphnia for experimental purposes has the advantage of being non-invasive because the beating insect heart can be viewed easily through the exoskeleton, and is non-destructive ie. dissections need not be carried out and so the organism can be released back to nature after it has assisted us with our work.

The drugs required to be used in this experiment are cardiac ion channel blockers, these act on different ion channels of the heart causing the cardiac potential to be affected.

## Cardiac Action Potential

Phase 4 as portrayed in the diagram shows the resting membrane potential.

Phase 0 is known as the rapid depolarization phase. The slope as shown in the diagram portrays the rate of depolarization of the cell and is known as dV/dtmax. At this phase the Sodium Channels open which caused a rapid influx of Sodium ions into the cell.

The ability of the cell to open the Sodium channels is to do with the membrane potential when it has become excited. At baseline -85mV the Sodium ion channels are not open, however when excited they open, causing an influx of sodium ions.

If the membrane is less negative, the fast sodium ions become inactive and insensitive to opening, and so this leads to a slight response to excitation of the cell membrane thus leading to a lower Vmax. If the resting membrane becomes too positive then sodium channels will not be able to flow through effectively and so will cause the delay of conduction through the heart. This can cause an increase in arrhythmias.

Phase 1 takes place with the inactivation of fast Sodium Channels. Chloride ions move across the cell membrane and therefore this causes a change in membrane potential, from the Potassium ions,

Phase 2 otherwise known as the ‘ plateau’ phase of the cardiac action potential is maintained by an inward movement of calcium ions and an outward movement of Potassium ions.

Phase 3 known as the rapid repolarisation phase, the action potential of the Calcium channels close and the postassium channels at this point are still open, this therefore enables a net outward current which causes a negative charge in membrane potential, this causes the potassium channel to open and the net outward of Potassium channels causes the cell to repolarise. The potassium channel close when the membrane potential is back to -85mV.

The drugs to be used on the daphnia heart are channel blockers and therefore may affect the cardiac action potential due to the blocking of certain channels, the drugs that are to be applied to the daphnia heart are:

## Lidocaine

Lidocaine is a Class Ib antiarrhythmic agents and are known as sodium channel blockers. They have a fast onset and offset kinetic which means the drug is more effective on faster heart rates. The drug has the ability to shorten the action potential duration and reduce the refractory period. The Vmax decreases in depolarised cells with fast response action potentials due to the drug. The action potential duration either goes unchanged it may decrease. File: Action potential Class Ib. svg

The effect of Class Ib drugs on the cardiac action potential

Class Ib drugs are generally more specific towards voltage gated Sodium Channels, lidocaine blocks the Sodium channels in their open and inactive states and does have the ability to bind in the resting state.

Lidocaine acts by the inhibition and conduction of nerve impulses by decreasing the neuronal membranes permeability to sodium ions; this prevents depolarisation from occurring and so results in a blockade of conduction.

## Glibenclamide

Glibenclamide is a sulfonylurea that acts on Type II diabetes, at nanomolar concentrations, the drug binds to the sulfonylurea receptor (SUR) which are found on β – Pancreatic cells, the drug causes the inhibition of ATP sensitive Potassium channels and this causes insulin to be released and therefore secreted into the blood.

Glibenclamide is also used as a CFTR Chloride channel blocker, it is voltage dependant and the drug acts by competing with Chloride ions for a common binding site within a large intercellular vestibule part of the CFTR pore.

## Verapamil

Verapamil is an L – type Calcium channel blocker. The drug acts by blocking the voltage gated calcium channel in cardiac muscle and therefore causes a decrease in cardiac contractility.

In cardiac pharmacology, calcium channel blockers can also be classified as IV antiarrhythmic agents. As calcium channels are concentrated in the sinoatrial (SA) and atrio-ventricular nodes (AV), these agents can be used to decrease impulse conduction through the AV node, and therefore slow the heart rate down.

## 4- Aminopyridine

4 – Aminopridine is a Potassium channel blocker also known as a Class III agent; this therefore prolongs the process of depolarisation.

Effect of Class III agent on cardiac potential

Since the Class III agent does not correlate with the sodium channel then conduction velocity does not decrease. Prolongation of the action potential, duration and refractory period along with normal conduction velocity, causes re – entrant arrhythmias from being prevented.

## Carbenoxelone

Carbenoxelone is a gap junction blocker and acts by blocking the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) they block the connexion channels and gap junctions.

The use of the drugs (as mentioned above) are to demonstrate whether the daphnia heart will respond to the drugs as we expect them to ie. how the mammalian heart would respond. Depending on how well the results from the daphnia heart correlates with what we know from the mammalian heart, determines the degree of usefulness of the daphnia in educational purposes.

## Aims and Objectives

The purpose of the experiment was to determine the presence of the principle voltage gated ion channels within the daphnia heart. The use of cardioactive drugs to inhibit various channels would indicate the existence of the receptor sites contained by the daphnia heart.

Cardioactive drugs being used:

ï‚· Lidocaine – Sodium Channel Blocker

ï‚· Glibenclamine – Chloride Channel blocker,

ATP sensitive Potassium Channel Blocker

ï‚· 4 – aminopyridine – Potassium Channel Blocker

ï‚· Verapamil – Calcium Channel Blocker

## Method

## Procedures for Safety

Since this was a laboratory experiment, we had to ensure safety within the lab. The laboratory that we carried out our experiment in was well ventilated, this was to minimise any inhalation of the drug or its fumes. To prevent ingestion of any material, food and drink was strictly prohibited within the lab.

Lab coats, goggles and gloves were worn in order to prevent contact of the cardioactive drugs with the skin or the eyes. It was advised that we use the drugs promptly and when we no longer required it, to put it back in the packaging and to the side.

A COSHH and biological safety form was filled out for all of the four cardioactive drugs, this was to ensure that the safety guidelines for each of the four drugs were known prior to the experiment.

## Control Experiment

Twenty daphnia were pipette from a bag containing daphnia, pond water and residue. These daphnia were placed into individual, conical bottomed multiwell plates. Excess water was removed from each well using a pipette with a thin tip, this was done to prevent the daphnia from moving excessively and therefore being able to see clearly the heartbeat of the daphnia.

Once the process was completed with twenty daphnia, a Lympus CK-40 light microscope was used to observe the heartbeat, settings on the microscope required adjusting in order to see a precise image of the heart beat. An image analysis software known as ‘ Mirovideo Capture’ was used in order to record the heartbeat of individual daphnia 3 times for 10 seconds each. The 10 second videos were played in slow motion, in order to count the number of beats per ten seconds. An average of the three values were calculated and then multiplied by 6 in order to find the beats per minute.

This experiment was carried out in order to see whether the group mean heart rate varied with an increasing sample size.

Experiment on varying temperatures.

In order to see whether temperature had an effect on the heartbeat we put the daphnia at temperatures of:

* 6-8 °C
* 21°C (Room Temperature)
* 37°C
* 6-8 °C

Daphnia were put in the fridge to achieve a temperature of 6 – 8°C. Six daphnia were then placed into individual wells (procedure as in Control Experiment). The microscope was used to observe the heart beats and three recordings were made for each daphnia. An average of the three values were calculated and then multiplied by 6 in order to find the beats per minute.

21°C (Room Temperature)

The daphnia were stored in room temperature and the experiment above (6-8 °C) was repeated for this temperature.

37°C

The daphnia were stored in an incubator at 37°C and the experiment above (6-8 °C) was repeated for this temperature

## Control experiment using Ethanol

From the cardioactive drugs, Lidocaine and Glibenclamide were required to be dissolved in ethanol, before they could be made up into a stock solution, www. tocris. com was visited to find the following information. Six daphnia were therefore pipette into individual wells, a 10% solution of ethanol was added to each daphnia and set aside for 10 minutes, this was to give time for the solution to have its effect, if any on the daphnia. The average heartbeat of each daphnia was then recorded to see if there was any alteration in heart rate.

The same experiment was repeated with a strength of 1% ethanol.

## Control experiment using distilled water

An experiment was carried out in order to see whether the daphnia were able to survive in distilled water, firstly the daphnia were placed in six wells and their hearbeat was recorded, the six daphnia were taken from their original environment of pond water and replaced with distilled water. The daphnia were left to swim in the distilled water over night. The heart beats of the daphnia were then recorded to see if they were any different to that of the pond water, results showed that there was no difference and therefore distilled water too could be used to make up stock solution.

## Procedure for Application of drugs.

Prior to the drugs being added, heart rates of the six individual daphnia were recorded three times.

Stock solutions were made up by weighing out xg on an accurate weighing balance (4 decimal places), the drug was poured into a 10ml conical flask, 1ml of ethanol was added to the solution (for Lidocaine and Glibenclamide only), this was then brought up to the base of the meniscus using part pond water and part distilled water. Six daphnia were placed in welled plates, excess water was removed and the drug solution at x% was added, they were placed aside for 10 minutes, this was in order to ensure enough time was given for pharmacological effects of the drug to take place on the daphnia heart.

## Experiment using Lidocaine

As there was uncertainty as to the strength that would have an effect on the daphnia heart, a standard solution of 1g in 100ml (0. 1%) was made up. Since the 0. 1% solution had a profound effect on the heart beat it was used as a stock solution and so it was diluted down to form the rest of the strengths in order to achieve a dose response curve.

At a strength of 0. 1% it was found that the lidocaine stopped the heartbeat of the daphnia, therefore diluents of the 0. 1% solution were made up:

* 0. 05% solution – 5ml of stock made up to 10ml (with pond and distilled water)
* 0. 02% solution – 4ml of 0. 05% solution made up to 10ml
* 0. 01% solution – 5ml of 0. 02% solution made up to 10ml
* 0. 001% solution – 1ml of 0. 01% solution made up to 10ml

Each solution of varying concentrations were tested on the daphnia using the same procedure as above, each solution was left for 10 minutes in order to see the effects on the daphnia heart. Diluents were continually made until they had no effect on the heart.

## Experiment using Glibenclamide

A concentration of 0. 1% was made up in order to see the effects on the heart, it was found at this strength there was little if no effect to the heart, therefore with the aid of trial and error it was found that at a strength of 0. 3% the rate of the heart decreased, however it did not limit it completely. We therefore tested the daphnia at drug concentrations of 0. 5% and it seemed to have the same effect as the strength at 0. 3%, we therefore used this strength as the stock solution and made diluents from it:

* 0. 3% stock solution – 0. 03g glibenclamide dissolved in 1ml of 5mM ethanol and made up to 10ml
* 0. 25% solution – 7. 5ml of stock solution made up to 10ml
* 0. 2% solution – 8ml of 0. 25% solution made up to 10ml
* 0. 1% solution – 0. 01g of glibenclamide made up to 10ml (original)

The different strengths were applied to the daphnia (procedure as mentioned before) and the results for each concentration were obtained.

## Experiment using verapamil

The standard solution of 0. 1% was made, though this solution had an effect on the heart it was not a profound effect, therefore the daphnia were tested at drug concentrations of 0. 2% which ultimately stopped the heart beat completely, therefore it was used as a stock solution and diluents were made from it:

* 0. 2% stock solution – 0. 02g verapamil dissolved in 10ml
* 0. 15% – 7. 5ml of stock solution (0. 2%) made up to 10ml
* 0. 1% – 0. 01g of verapamil made up to 10ml (original)
* 0. 01% – 1ml of original (0. 1%) made up to 10ml

These varying strengths were added to the daphnia (procedure as mentioned before) to see the effect on the heart.

## Experiment using 4-aminopyridine

The standard solution of 0. 1% was made and from it its diluents. At 0. 1% the heart rate was affected so a solution of 0. 5% was made to see if any further changes to the heart occurred.

* 0. 5% solution – 0. 05g of 4-aminopyridine made up to 10ml
* 0. 1% stock solution – 0. 01g of 4 – aminopyridine made up to 10ml
* 0. 01% solution – 1ml of stock solution made up to 10ml
* 0. 001% solution – 1 ml of 0. 01% solution made up to 10ml

Each strength were added to the welled plates containing daphnia (procedure as mentioned before). to see the effect on the heart.

## Experiment using Carbenoxelone

As researched we found that a small weight of carbenoxelone was required to effect the heart rate, we therefore decided to stick with the standard strength of 0. 1%, this had no effect on the heart and so we made up a strength of 0. 2% which too had no effect on the heart, we therefore decided that it would not be worthwhile to use more drug, since the concentrations had no impact on the heart. The solutions made therefore were:

* 0. 2% solution – 0. 02g of carbenoxelone made up to 10ml
* 0. 1% solution – 0. 01g of carbenoxelone made up to 10ml

Each varying strength of drug was added to individual daphnia (procedure as mentioned before).

## Results

## Discussion.

## Control Experiment

The control experiment was carried out in order to see whether the group mean heart rate varied with an increasing sample size, the results show that the standard deviation was within 10% of the mean, 7. 96% to be precise, therefore this method was acceptable for use, since the heart beats did not vary much.

We therefore adopted this method and lowered the number of daphnia from 20 to 6, since 6 was a sufficient number for accurate results.

## Dose response curve of Lidocaine

The results show that the application of Lidocaine on the daphnia heart caused a dramatic increase in percentage change in heart rate at a strength of 0. 1%, experimentally it was found that within 20 – 30 seconds the daphnia heart completely stopped beating. This is since Lidocaine is a Sodium channel blocker and acts by impairing the conduction of Sodium ions through sodium channels. Therefore at a strength of 0. 1% the drug was adequate to cause a 100% percentage change in heart beat.

By diluting the strength to 0. 05% the drug decreased the percentage change in heart rate compared to 0. 1% however it did not completely stop the heart nor did it affect the heart tissue, the percentage change was 38%.

At a strength of 0. 001% the drug had little to no effect on the heart

The general trend of the graph shows that when increasing the concentration of the drug, there is a general increase in the percentage change in heart rate which means that the heart rate is slowing down.

## Dose response curve of Glibenclamide

The graph shows that at a concentration of 0. 3% of Glibenclamide the drug had a mild effect on the heart, it caused a 30 percent change in heart rate and therefore though this was a high concentration of drug it did have a minimal effect

At a strength of 0. 25% of Glibenclamide, the effect of the drug fell by 50%, showing that the percentage change in heart rate was about 15%.

At a strength of 0. 1% of Glibenclamide there was little to no change in heart rate, showing that the drug had no effect on the heart.

The general trend of the graph shows that with an increase of drug there was increase in percentage change of heart rate, which means the more concentrated the drug the more effect it had on the heart in terms of slowing the heart rate down.

Since the drug inhibits the CFTR

## Dose response curve of Verapamil

Verapamil was the most potent drug out of all the cardioactive drugs. At a concentration of 0. 2% it caused a 100% change in heart rate which means it stopped the heart beat completely.

At 0. 1% it still had a dramatic impact in terms of slowing the heart rate down, there was a 75% change in heart rate which shows at this concentration, though the hearts had not fully stopped, though they did decrease dramatically.

At a concentration of 0. 001%, there was little to no change in heart rate, showing that the drug, at this strength, had no effect on the heart.

Experimentally, we found that the drug also affected the way the heart contracted ie. the movement, see pictures below:

(put in pictures and explain)

## Dose response curve of 4 – amino pyridine

In order to achieve a dose response curve for 4 – amino pyridine we required a large range of values from 0. 001% to 0. 5%

The graph shows that at 0. 5% there was a 100 percent change in heart rate, this shows that at this concentration the drug was more potent and stopped the heart beat completely

At a concentration of 0. 01% the drug had a little to no effect on the heart rate.

The general trend of the graph is – the greater the concentration of 4 – amino pyridine the greater the percentage change in heart rate.

## Dose response curve of Carbenoxelone

The addition of carbenoxelone had no effect on the daphnia heart, this is portrayed from the graph as there is a straight line at concentrations 0. 2% and 0. 1%, therefore the drug had no impact on the heart rate.

This may be due to one of two reasons, either there are no gap junctions present in the daphnia heart, or the carbenoxelone does not have an effect on the innexins (found in daphnia heart), therefore only connexins (found in human heart).

## Conclusion

Upon analysis, it can be seen that the drugs Lidocaine, Verapamil and 4 – Aminopyridine slowed the heart rate down, this shows that Sodium channels, Potassium channels and Calcium channels are present in the heart of the daphnia.

Though the heart rate of the daphnia did slow down when Glibenclamide was added to it, it did not decrease dramatically. There is no proof, therefore, to suggest that there are chloride ions present in the daphnia heart, this is since glibenclamide can be used as a chloride channel blocker, but it also inhibits ATP sensitive Potassium channels, therefore further research needs to be done in order to see whether the daphnia heart contains chloride ions. The use of another drug that specifically blocks chloride channels, seems like a good alternative.

The drug, carbenoxelone shows that there may not be any gap junctions present in the daphnia heart, therefore an alternative method may be used for intercellular signal transmission.

Overall the daphnia could be used to show the effects of the Lidocaine, Verapamil and 4 – Aminopyridine on the heart, therefore the daphnia heart is a useful educational tool, to show the effects of drugs on the heart. The fact the daphnia is one cell thick and transparent makes it straightforward to find the heart under the microscope. Therefore the daphnia can be used for educational purposes in undergraduate study.

## Further Studies

An ECG study can be carried out on the heart of the daphnia by using probes to touch the daphnia heart. The ECG study can be used in order to interpret the electrical activity of the heart. From the results we would be able to analyse the ion channels and at what point ions leave and enter the membrane, from this we are able to see how closely related the daphnia heart is to the mammalian heart.