Introduction:
Considering the vitality of the human heart and the prominence of Cardiac related diseases in modern society, extensive biomedical research has been devoted to understanding the functioning of the heart. Specifically, in the last fifty years biophysical insights has led to understanding of the electrical basis of myocardial contraction(Tripathi, Ravens, & Sanguinetti, 2011).

The heart beat is known to originate from the pacemaker cells of the sinoatrial node(SAN). The SAN controls the heart rate by initiating rapid and synchronized depolarization of the heart. (Tripathi, Ravens, & Sanguinetti, 2011) The action potentials within the cardiac cells are controlled and generated by a variety of ion gradient. Specific ion channels facilitate the movement of Na^+, K^+, Ca^{2+},and Cl^- down their concentration gradients. It is this ion movement that allows the maintenance of the resting potential and production of an action potential (Tripathi, Ravens, & Sanguinetti, 2011).

This cardiac muscle contraction is dependant on calcium induced calcium release. This is resultant from the propagation of the action potential (initiated by the SAN) travelling along the surface membrane and down the T-tubules which activates L-type Ca^{2+} channels. This results in a movement of Ca^{2+} into the cardiac myocyte. This increase in Ca^{2+} concentration is sensed by the ryanodine receptors in the sarcoplasmic reticulum membrane which causes these receptors to efflux Ca^{2+} (Yue-Kun & Allen, 1999). These ions then bind to troponin C which shifts the troponin complex making it possible for the myosin head to bind to the actin filament. The myosin head then undergoes ATP hydrolysis which causes the thick filaments to slide past the thin resulting in muscle tension. The Calcium is eventually removed and the tension is alleviated. (Boulpaep & Boron, 2009) Evidently Ca^{2+} is a detrimental ion to myocardial contraction and a vital signaling molecule. Ca^{2+} is utilized by certain stimulants, like β-adrenergic stimulants, to achieve physiological responses.

β-adrenergic stimulants are known to cause inotropic effects on the mammalian cardiac cells. (Valentinia & Parati, 2009)The affects of these stimulants with regards to amphibian cardiac cells are of interest. The amphibian heart is known to be distinct from the mammalian heart for several reasons. While not only does it only have one ventricle, the primary β-adrenergic stimulant of the toad in adrenaline (as opposed to noradrenaline in the mammalian heart) (Department of Physiology, University of Minnesota, , 1965). For this reason adrenaline is one of the focuses of this study. It is anticipated that adrenaline will have similar affects on amphibian cells as noradrenaline on mammalian myocardial cells.

β-adrenoceptors are G protein-coupled receptors that are utilized by the Sympathetic Nervous System (SNS) and thus targeted by the Catecholamine (i.e. adrenaline and noradrenalin). (Dale & Rang, 2011)There are three separate types of β-adrenoceptors . β1-adrenoceptors are the receptors responsible for causing an increase in heart rate and contractile force and of particular interest. B1-adrenoceptors are generally located in the heart and kidneys. β2-adrenoceptors and β3-adrenoceptors are found primarily in the lungs adipose tissue respectively. (Dale & Rang, 2011)

Adrenaline induces the inotropic effect by binding to the β1-adrenoceptors. This results in an increase in cAMP concentration as a second messenger which activates protein kinase A. L-type Ca^{2+} channels are then phosphorylated as a result of this causing an efflux of Ca^{2+} from the sarcoplasmic reticulum. (Dale & Rang, 2011) Studies have found that the 'firing rate' of the SAN (which sets the heart rate) is only increased when the concentration of ca ions is elevated in amphibian cells. (Yue-Kun & Allen, 1999) Adrenaline increases heart rate by decreasing the delay at the AV node and decreasing K permeability. This means there is a more rapid drift to threshold

and increased depolarization rate. This decreases the time required for excitation and thus increases heart rate. (Boulpaep & Boron, 2009)

Propranolol is a known β-adrenoceptor antagonist. Propranolol is known to have little effect on heart rate and cardiac output on mammalian cells. It blocks β1 and β2 receptors, which prevents the adrenaline from binding and thus from eliciting any inotropic effects of myocardial cells. (Dale & Rang, 2011)

Studies conducted on amphibian myocardial cells have indicated that adrenaline elicits a similar physiological response on the amphibian heart as it does to the mammalian (i.e. increasing heart rate and cardiac output). There is however some conflict in the findings for the effects of Propranolol. It is widely believed that Propranolol, when administered in conjunction with adrenaline, merely negates adrenaline by blocking β receptors. Some recent studies have, however, shown that in amphibian myocardial cells, Propranolol can have antihypertensive action (i.e. reduce cadiac outpute). (Hillman, 1981)

For that reason it is anticipated that in this study the application of adrenaline to a Bufo marinus heart will result in increased heart rate, while the application of Propranolol in conjunction with adrenaline will result in a heart rate lower than the heart rate induced by adrenaline alone. It is the intention of this study to determine the effects of these β-adrenergic stimulants on amphibian myocardial cells

Hypothesis:
The application of the adrenaline solution will increase the heart rate of a cane toad (Bufo marinus) with comparison to heart rate without drug administration. The application of Propranolol prior to adrenaline will cause heart rate by the toad heart to be less than that induced by adrenaline alone.

Method:
Subjects:
A double-pithed bufo marinus was placed ventral side up on a dissection board. A longitudinal and lateral incision was made across the abdomen of the toad and the sternum was removed to expose the thoracic cavity. The pericardium was cut away and the heart was lifted out of the cavity. The heart was attached via a hook through the apex to a force transducer, suspended above the toad on a retort stand. Care was taken to position the toad heart directly below the force transducer and the transducer was raised to ensure the string was taught.
Protocol
The readings of the force transducer heart were then recorded in Labchart in mN. Once the heart rate was deemed to be consistent a three minute long baseline was recorded, and ten measurements of heart rate were taken at various points. Heart rate was calculated by observing the number of cycles completed over a fifteen second period and multiplying by four to determine the number of beats per minute. During this period the heart was intermittently rinsed with frog ringer solution. Three drops of 1mM adrenaline solution were then administered to the heart using a plastic pipette. After allowing waiting sixty seconds for the adrenaline to take effect, the heart rate was found at ten different time periods. The heart was then rinsed with frog ringer solution and once the heart rate was adjudged to have returned to baseline, a second three minute long baseline was recorded, and heart rate was calculated for ten different periods. Three drops of a 1mM Propranolol solution were then administered to the heart and after a period of one minute three drops of the adrenaline solution were added. One minute was waited post administration to allow the drugs take effect prior before heart rate was calculated for 10 different time periods.

Data Analysis:
Data was then analysed and graphed in GraphPad Prism using a 1-way ANOVA with Tukey's post test. Both the mean and the standard error of the mean(SEM) were calculated during this process and significance was determined at $P < 0.05$.

Results:
Results demonstrated that there was a significant increase in the B. marinus heart rate following the administration of the adrenaline solution compared to the heart rate prior to the application of any drugs (mean increase of 7.240 bpm, $P<0.001$, see figure 1). There was also a more substantial decrease in heart rate post the staggered administration of Propranolol and adrenaline with respect to the initial heart rate (mean decrease 18.64 bpm, $P<0.001$, see figure 1)
Figure 1:

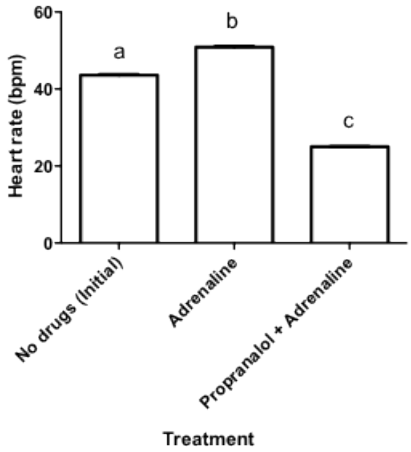

Fig. 1: A graph displaying the mean ± SEM heart rate of the Bufo Marinus heart (n=1) during normal conditions (no drugs), during treatment with adrenaline (3 drops of 1mM solution) and during treatment with propranalol and adrenaline (each 3 drops of 1mM solution). Letter code above a column denotes a signficant difference ($P<0.05$) from other groups.

Discussion:
The results of this study indicate that, as anticipated, adrenaline increased the heart rate of the toad with respect to the initial heart rate reading prior to drug administration. When the Propranolol was added in conjunction with adrenaline a substantial decrease was seen in the heart rate of the B. marinus. While it was predicted that the Propranolol would negate the effects of the adrenalin, it was not anticipated that it would result in such a substantial decrease in heart rate relative to the initial reading taken prior to drug administration.

The results regarding adrenaline demonstrated that the sympathetic transmitter (adrenaline) increased the heart rate by interacting with the β-adrenoceptor. This resulted in the modulation of the ion channels involved in producing the activity of the SAN i.e. the L-type calcium current ICa(L) the delayed rectifier current IK and the hyperpolarization-activated cation channel. (Yue-Kun & Allen, 1999)As previously established, binding of adrenaline to the β-adrenoceptor triggers a pathway that eventually results in the increase of Ca^{2+} concentration in the sarcoplasmic reticulum. Studies have shown that the increased activity in the pacemaker cells can be attributed to both the modulation of the ion channels and the increase in the calcium ion concentration. (Yue-Kun & Allen, 1999)

While the increase in heart rate of the toad after adrenaline was administered was deemed substantial, it was still anticipated that this increase would have been more significant. It is postulated that due to seasonal variation in responses to adrenaline,

the toad would have been less sensitive to the drug. It is known that there is a notable difference in the constriction of the vasculature depending on seasonal variation. Specifically, adrenaline elicits a response, on average, three times greater in summer than the response in winter. (Morris, 1982) It is theorized that this difference is triggered by subtle conformational changes in the receptors that takes place in summer in response to hormones associated with breeding. (Morris, 1982) Thus, as this study was undertaken in winter, there is a basis to believe that the effects of the adrenaline on heart rate were minimized due to the sensitivity of the receptors.

The significant decline in heart rate post the application of the adrenaline and Propranolol was not entirely anticipated. As established, Propranolol is known to be a β-adrenoreceptor antagonist. This means that it competitively binds to the β-adrenoreceptors, effectively blocking the adrenaline from binding and eliciting a response. (Dale & Rang, 2011) For that reason it was anticipated the combination of adrenaline and Propranolol would result in a heart rate similar to the initial reading where there was no drug application. Under normal circumstances Propranolol can alleviate hypertension and reduce heart rate by competitively binding to the adrenoreceptors which inhibits the influence of the SNS. (Dale & Rang, 2011) In this circumstance, as the toad was pithed (meaning the autonomic nervous system was disabled) there shouldn't have been a significant decrease as the sympathetic nervous system was not innervating the pacemaker with adrenaline.

If anything, a slight increase in hear rate, relative to the baseline would have been anticipated considering Propranolol is a non-selective β-adrenoceptor, meaning it will bind to both β1 and β2 adrenoreceptors. While adrenaline primarily acts on β1-adrenoreceptors to influence heart rate, evidence indicates that it also acts on receptors that are neither α-or β-receptors which contributes to the myocardial excitement. (Morris, 1982) Considering that Propranolol only blocks the beta receptors it would be anticipated that there would be a small increase in heart rate caused by these ambiguous receptors.

While the heart rate results post proporanolol and adrenaline administration appear anomalous there still are some plausible justifications for this outcome. Some studies have found proporanolol to have a relaxation effect on the heart. Specifically a study from by Portland State University found evidence to indicate that a Propranolol injection into toads decreased exercise heart rate in a dose-dependent manner. (Hillman, 1981)

Also, when considering that the Propranolol and adrenaline treatment was conducted last, it is possible that the low heart rate could be the result of stress and the degeneration of the heart. Particularly when considering that this trial was conducted after the adrenaline trial. Throughout the adrenaline trial the heart could not be rinsed with frog ringers solution for obvious reasons so potential damage could have occurred to the heart if it 'dried out' during this period.

Seasonal variance may also have been a contributing factor to this anomaly. While toads can be less responsive to certain drugs in winter they are also known to suffer from inconsistent and fluctuating heart rates. (Morris, 1982) This could have contributed to the low heart rate for the Propranolol.

Overall the results from this experiment verified the hypothesis as it was anticipated that the application of the adrenaline solution would increase the heart rate of a B. marinus with comparison to heart rate without drug administration. While the results for the application of Propranolol prior to adrenaline were mildly anomalous, as predicted the drug applications caused heart rate by the toad heart to be less than that induced by adrenaline alone.

Bibliography

1. Boulpaep, E. L., & Boron, W. F. (2009). *Medical Physiology*. Philadelphia: Elsevier.
2. BROWN, H. F., DIFRANCESCO, D., & NOBLE, S. J. (1979). How does adrenaline accelerate the heart? *Nature* , 235-236.
3. Dale, & Rang. (2011). *Pharmacology*. Edinburgh: Elsevier.
4. Department of Medicine, The University of Queensland. (n.d.). (-)-Adrenaline elicits positive inotropic, lusitropic, and biochemical effects through β2-adrenoceptors in human atrial myocardium from nonfailing and failing hearts, consistent with Gs coupling but not with Gi coupling. *Naunyn-Schmiedeberg's Arch Pharmacol* , 11-28.
5. Department of Physiology, University of Minnesota, . (1965). Adrenergic mechanisms in the bullfrog and turtle. *American Journal of Physiology , 209*, 1287-1294.
6. Hillman, S. S. (1981). Effects of DL-Propranolol on exercise heart rate and maximal rates of oxygen consumption in Scaphiopus intermontanus 1. *CELLULAR AND MOLECULAR LIFE SCIENCES* , 940-941.
7. Morris, J. L. (1982). Seasonal variation in responses of the toad renal vasculature to adrenaline . *NAUNYN-SCHMIEDEBERG'S ARCHIVES OF PHARMACOLOGY , 320*, 246-254.
8. Petroff, M. e. (1994). Lusitropiceffectsofct-and[-adrenergicstimulation in amphibian heart. *Molecular and Cellular Biochemistry* , 87-95,.
9. Tripathi, O., Ravens, U., & Sanguinetti, M. (2011). *Heart Rate and Rhythm*. Berlin: Springer.
10. Valentinia, ,. M., & Parati, G. (2009). Variables Influencing Heart Rate. *Progress in Cardiovascular Diseases* , 11-19.
11. Yue-Kun, J., & Allen, D. G. (1999). How does â_adrenergic stimulation increase the heart rate? The role of intracellular Ca¥ release in amphibian pacemaker cells. *Journal of Physiology* , 793—804.