Introduction:

The physiology of cardiac function is an area, which has been studied extensively throughout the history of anatomical science. Cardiac function, especially in mammalian subjects, has been the focal point of many experiments; however the amphibian heart has also been an area of investigation. An aspect of cardiac function, which has come under particular exploration in both mammalian and amphibian models, is heart rate specifically with regard to adrenergic stimulation and inhibition. This report will investigate these adrenergic effects on heart rate in the amphibian heart specifically that of the cane toad (*Bufo marinus*).

The rhythmic contraction of the heart is an intrinsic property of cardiac muscle and is measured in terms of heart rate (DiFrancesco, 1993). Specialised pacemaker cells within the heart control heart rate (DiFrancesco, 1993). These myocytes have the ability to spontaneously depolarise and fire off action potentials (Boron and Boulpaep, 2009) even when separated from other cells, and thus give rise to the inherent beating or pacemaker activity of the heart (DiFrancesco, 1993). The firing rate of a small cluster of such pacemaker myocytes, located within the sinoatrial (SA) node in mammals and sinus venosus in amphibians, sets the timing for contractions and thus determines heart rate (Ju and Allen, 2001).

Despite obvious structural differences between mammalian and amphibian hearts, amphibians have a three-chambered heart with two atria and a ventricle (Gentz, 2007) unlike the four-chambered heart of mammals, the means of heart rate regulation appear quite similar. In both cases the pacemaker potential, a spontaneous period of diastolic depolarisation, is known to initiate the firing of action potentials by pacemaker cells (Ju and Allen, 2001). This pacemaker potential and in turn the heart's automaticity, are principally controlled by three voltage-gated membrane currents, $I_{Ca(L)}$, I_K and I_f (Boron and Bouelpaep, 2009). However cane toad pacemaker cells lack I_f thus demonstrating that other currents including the Na^+–Ca^{2+} exchange current, T-type Ca^{2+} current and background Na^+ current are involved in pacemaking (Ju and Allen, 2001). As such, the intracellular concentration of Ca^{2+} has a large influence on the firing rate of pacemaker cells. Other regulators of pacemaker activity include; sarcoplasmic reticulum (SR) Ca^{2+} release, which is needed for regular firing of pacemaker cells (Ju and Allen, 2001); autonomic stimulation, via the sympathetic or parasympathetic nervous systems, and circulatory substances such as catecholamines (e.g. adrenaline) which can alter heart rate (Kestin, 1993).

Adrenergic stimulation of the heart in both mammals and amphibians is known to affect heart rate. In cardiac muscle the application of noradrenaline in mammals, and adrenaline in amphibians, elicits an increase heart rate as a result of β-adrenergic stimulation (Cousins and Bramich, 1997). Both catecholamines are agonists of β-adrenoceptors and show little selectivity in the receptors they activate (Rang et al., 2011). Within the toad heart adrenaline acts on postjunctional β-adrenoceptors in the sinus venosus (like noradrenaline in the mammalian SA node) to elevate cyclic adenosine 5'-monophosphate(cAMP) levels (Bramich et al, 2001). This signals a transduction pathway, which causes the modulation of the voltage-gated ion channels involved in pacemaker activity via protein-phosphorylation and results in tachycardia (Bramich et al., 2001).

Positive chronotropy observed in the heart is associated with two principal changes to the pacemaker action potential. Firstly an increased rate of diastolic depolarization is observed and secondly there is an increased speed of repolarisation following the action potential (Cousins and Bramich 2004), both of which allow an increased firing rate of action potentials and thus an increase in the number of contractions per time period. It is also known that increased amplitude of systolic rise in intracellular Ca^{2+} concentration

($[Ca^{2+}]_i$ transients), as a result of β-adrenergic stimulation, increases firing rate with adrenaline administration, once again increasing heart rate (Ju and Allen, 2004).

However the affect on heart rate of β-adrenoceptor antagonists or β-blockers in combination with agonists such as adrenaline is also of interest. One such antagonist is propranolol, which acts by blocking β_1 and β_2-adrenoceptors (Rang et al., 2011). At rest propranolol has little affect on heart rate (Rang et al., 2011) and when applied in conjunction with adrenaline (β-adrenoceptor agonist) inhibits the effects of β-adrenergic stimulation (Ju and Allen, 2004). In cane toads, experiments have shown that propranolol administration can reverse the effects of adrenergic stimulation (via adrenaline application) on pacemaker activity by reducing by reducing $[Ca^{2+}]_i$ transients and firing rate back to baseline rates (Ju and Allen, 2004). As such this study aims to determine whether this affect is also observed when the propranolol is applied to the heart prior to adrenaline application.

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

Methods:
Subjects:
A double-pithed bufo marinus was placed ventral side up on a dissection board. Longitudinal and lateral incisions were 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 for the 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 contractile cycles (heart beats) completed within a fifteen second period and multiplying this number by four to determine the number of beats per minute. During this baseline period the heart was intermittently rinsed with frog ringer solution. Three drops of 1mM adrenaline solution were then administered to the heart using a 1.5mL plastic pipette. After allowing a waiting period of sixty seconds for the adrenaline to take effect, the heart rate was recorded at ten different time periods, using the above process. 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 during this timeframe (post experimentation the second baseline was analysed and was found to hold no difference to the original baseline, based upon 10 heart rate measurements). 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 adrenaline administration to allow the drugs take effect before heart rate was calculated for 10 different time periods (using aforementioned process).
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. Significance was determined at $P < 0.05$.

Results:

The observed mean heart rate data for each replication showed a significant increase in heart rate upon exposure to adrenaline, compared to baseline in the cane toad heart (mean increase of 7.240 bpm, $p<0.001$; see figure 1). The application of propranolol lead to an immediate and statistically significant decrease in heart rate (mean decrease 18.64 bpm, $p<0.001$). The addition of adrenaline did not have a noticeable effect on heart rate after propranolol was applied, and there was a statistically significant difference in heart rate between the adrenaline treatment and the treatment with propranolol and adrenaline applied together. It was observed that the effect size of the propranolol and adrenaline treatment was much larger than the size of the increase upon exposure to adrenaline alone (mean decrease of 18.64bpm for both drugs, mean increase of 7.240 for adrenaline alone; see Figure 1).

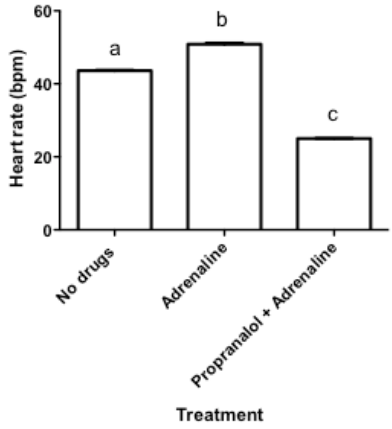

Figure 1: A graph displaying the mean ± SEM heart rate of the *Bufo Marinus* heart (n=1) during normal conditions (no drugs), during treatment with adrenaline (3 drops of 1mM solution) and during treatment with propranalol and adrenaline (each 3 drops of 1mM solution). Letter code above a column denotes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

Discussion:

The experimental findings supported the hypothesis of the study. The application of adrenaline resulted in a significant increase in heart rate generated by a cane toad heart compared with the heart rate generated without the application of drugs (Figure 1.). A decreased heart rate with the application of propranolol prior to adrenaline was observed compared with the heart rate produced by adrenaline application alone (Figure 1.). However despite such evidence supporting the hypothesis, some unexpected results were observed. The decreased heart rate upon application of propranolol and adrenaline compared with the treatment where no drugs were applied specifically was not predicted.

The results confirm that β-adrenergic stimulation of the heart, via adrenaline, increases heart rate in amphibians. This is consistent with previous knowledge and studies where application of adrenaline results in an increased firing rate (Ju and Allen, 2004). The increased firing rate causing the positive chronotropic effects is due to a mechanism by which adrenaline causes an increased rate of diastolic depolarization and an increased speed of repolarisation following the action potential by modulating ion channels in the cardiac cells (Ju and Allen, 2004). These events decrease the duration of single action potentials thus

allowing more action potentials per time period, which equates to increased pacemaker activity and therefore increased heart rate.

However despite the obvious increase in heart rate with β-adrenergic stimulation compared with the baseline levels, it was lower than the increases observed in other studies. Collected data gave an average increase in heart rate of 16.6% from baseline with adrenaline administration whereas in previous studies (Azuma et al., 1965) the average increase had been calculated at 27.4%, a much larger value. A possible reason for this decreased change is the effect of cold temperatures on the cane toad heart. Experimental research suggests that β-adrenroceptors in cane toad cardiac muscles undergo a functional change at low temperatures (Cheng et al., 1982). This functional change then causes decreased receptor functionality at low temperatures and a less noticeable effect of adrenaline on heart rate in cane toads (Cheng et al., 1982). As such it is probable that the low temperatures observed on the day of experimenting resulted in a decreased effect of adrenaline on heart rate compared to previous studies.

The fact that the heart rate was greater during treatment with adrenaline alone than treatment with propranolol and adrenaline, was consistent with researched literature, which suggested that, propranolol as a β-adrenoceptor antagonist would inhibit the effects of adrenaline stimulation (Ju and Allen, 2004) by blocking β-adrenoceptors (Rang et al., 2011). However the decreased heart rate with respect to baseline levels where no drugs were applied was unexpected. There are a few possibilities for this occurrence, principally dependent on propranolol's nature as a β-blocker, including the inhibition of Ca^{2+} uptake by the SR due to propranolol (Zchut et al., 1996). Inhibition of Ca^{2+} uptake leads to decreased stores of Ca^{2+} within the SR and thus decreased SR Ca^{2+} release, which has been shown to slow heart rate (Ju and Allen, 2001). Propranolol is also known to cause bradycardia (a slowed heart rate) as an adverse effect (Rang et al., 2011) and it is possible that this occurred in the experimental specimen. It is also possible that the toad's heart was failing which may have further exaggerated such negative chronotropic effects and led to an even lower heart rate.

This study investigated the effects of β-adrenergic stimulation and inhibition of the cane toad (Bufo marinus) heart by adrenaline and propranolol respectively. It was confirmed that adrenaline increased the baseline heart rate in toads and also discovered that propranolol can decrease the heart rate compared to baseline (no drugs applied) even if adrenaline is applied afterwards. Further studies over a range of temperatures are necessary in order to determine whether propranolol usually has this affect.

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