EFFECT OF ADRENALINE VERSUS PROPRANOLOL WITH ADRENALINE ON THE CARDIAC FUNCTION OF Bufo *marinus*

INTRODUCTION:

Within amphibious species, α and β adrenergic receptor signal transduction pathways produce cellular responses which regulate the physical properties of cardiac contraction events (Maxwell and Webb, 2008). The two categories of adrenergic receptors, α and β types, are differentiated based on the sensitivity to varying catecholamines (Benfey, 1977). Furthering this, each type is further divided into sub-categories dictated by the specific cellular response induced by differing activating ligands (Benfey, 1977). Considering this ligand specificity there is a catecholamine dependent discrepancy between cellular responses, for different receptor types (Ask, 1983). Therefore, the presence of differing receptor types will cause cardiovascular function (heart rate and ventricular contractile force) to vary when treated with catecholamines such as adrenaline and propranolol. Within scientific studies involving amphibian species such as the cane toad (Bufo *marinus*) there remains contention over which receptor subcategories exist within the heart. Analysing the effect of drug treatment on cardiovascular function provides further evidence for determining the receptor composition within the toad heart.

Adrenergic receptors have mechanistic responses to catecholamines which regulate the signal transduction pathway leading to cellular response. Agonist ligands act to initiate the G-protein coupled receptor signal transduction pathway causing the activation of the trimeric G-protein attached to the receptor (Maxwell and Webb, 2008). Adrenaline is an agonist ligand which binds to each of the receptors α_1, α_2, β_1 and β_2 inducing differing cellular responses (Ask, 1983). Research also suggests a sympathetic excitation of the heart through adrenergic receptors which are neither α or β (Morris et al. 1981). The stimulation of α_1 receptors, which are coupled to phosphoinositide hydrolysis, by adrenaline, results in the direct increase in myocardial contractility (ventricular contractile force) of the heart (Lazou, 2002; Seraskeris and Lazou, 2002; Aggeli et al., 2002). However, research suggests α_2 adrenergic receptors are not present in the amphibian heart (Ask, 1983).Within the toad heart the β_1 and β_2 receptors are not identical to those identified in mammalian species (O'Donnel and Wanstall, 1982). However, the combined β adrenergic receptors maintain a sensitivity to adrenaline and produce both chronotropic (effects that change heart rate) and inotropic (effects that change contraction force) effects (O'Donnel and Wanstall, 1982). Chronotropic effects are thought to be dictated by the changes in free calcium ion concentration (Ca^{2+}) within pacemaker cells (Ju and Allen, 1999). However, inotropic effects are regulated by the changes in free calcium ion concentration within cardiac muscle cells (Allen and Blinks, 1978). Additional to α and β receptors, the stimulation of sympathetic nerves via unidentified adrenergic receptors within the toad heart produce effects which increase both heart rate and contractile force (Morris et al., 1981). Considering all of the potential receptors stimulated, the holistic organism physiological response is a product of the combined individual receptor responses.

In contrast to agonist ligands, certain catecholamine molecules competitively bind to receptors and produce a conformational change which does not initiate activation (Maxwell and Webb, 2008). These ligands are antagonists as the binding of such catecholamines interferes with the binding of agonists through competitive inhibition (Morris et al., 1981). This effectively limits the agonist induced activation of cellular receptors. Propranolol is an antagonist ligand which binds to β_1 and β_2 receptors (Benfey, 1977). The binding of propranolol to these β receptors prevents the binding of adrenaline and therefore the responses to the agonist are reduced (Maxwell and Webb, 2008). The inhibition of β receptors prevents these receptors from producing a cellular response in the form of an

increase in heart rate and contraction force. However, in the heart of amphibious species, the competition is not based purely on simple inhibition (Morris et al., 1981). Consequently, the sympathetic and α adrenergic receptors will still have an influence on the cardiac function of the heart in the presence of propranolol and adrenaline.

HYPOTHESIS:
In the presence of adrenaline (an agonist), heart rate and ventricular contractile force in a cane toad, *Bufo marinus*, will be greater than treatment with both propranolol (an antagonist) and adrenaline.

METHODS:
Protocol: A double pithed toad was dissected along the ventral surface of the chest to expose the heart. The heart was then attached to the force transducer using a pin through the apex of the ventricle. To determine changes in cardiac activity (heart rate and ventricular contractile force) in this experiment, the force transducer was connected to the data acquisition hardware Power Lab and cardiac function was recorded via the software program Lab Chart. Cardiac activity with no drug applied to the heart (control treatment) was recorded for 2 minutes. Frog ringer solution was applied to the heart for 3-4 seconds to ensure it did not desiccate. 3 drops of adrenaline at a concentration of 1mM was applied topically, using a 1.5mL pipette, to the heart of the toad. The force trace was allowed to stabilize for approximately 1 minute and then cardiac activity was recorded for 2 minutes. The heart was then washed with frog ringer solution until heart rate returned to baseline. 3 drops of propranolol at a concentration of 1mM was then topically applied to the heart using a 1.5mL pipette and cardiac activity was allowed to stabilize for approximately 1 minute. 3 drops of adrenaline at a concentration of 1mM was applied and cardiac activity was allowed to stabilize for approximately 1 minute. Cardiac activity was recorded for 2 minutes.

Data Analysis: Data for heart rate and ventricular contractile force under all treatment was extracted from the force trace in LabChart. Mean contractile force was determined from the difference between resting and peak ventricular force, from eight cardiac cycles. Mean heart rate was determined in beats per minute, by the number of ventricular contractile peaks over 4, 30-second blocks of recording for each treatment. The precision of the data was determined by the calculation of the standard error measurement (SEM). A one-way ANOVA with Tukey's post-hoc test was used to determine the effect of drug treatment on cane toad heart rate and ventricular contractile force with an accepted significance of $P<0.05$.

RESULTS:
Within the experimental investigation there was a significant difference in heart rate and ventricular contractile force between treatments. Treatment with adrenaline caused the heart rate to be significantly greater than the baseline (control) rate (see Figure 1). However, following the combined treatment of propranolol and adrenaline, the heart rate was significantly lower than the rates obtained for both adrenaline and baseline treatments (see Figure 1). For treatment with adrenaline, the contractile force was significantly smaller than the baseline force (see Figure 2). However, following the combined treatment of propranolol and adrenaline, the contractile force was significantly smaller than the force obtained for both adrenaline and baseline treatments (see Figure 2).

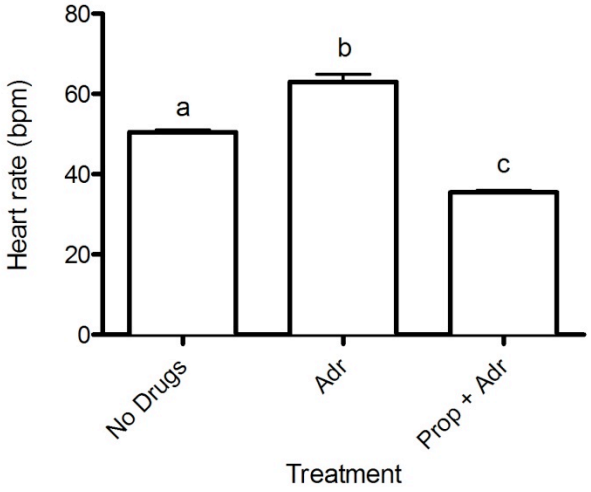

Figure 1: Mean + SEM heart rate (bpm) of the cane toad (*Bufo marinus,* n=1*)* under treatment with no drugs, adrenaline (3 drops, 1mM), propranolol (3 drops, 1mM) and propranolol + adrenaline (3 drops each, 1mM). Different superscript letters denote significant differences at $p<0.05$.

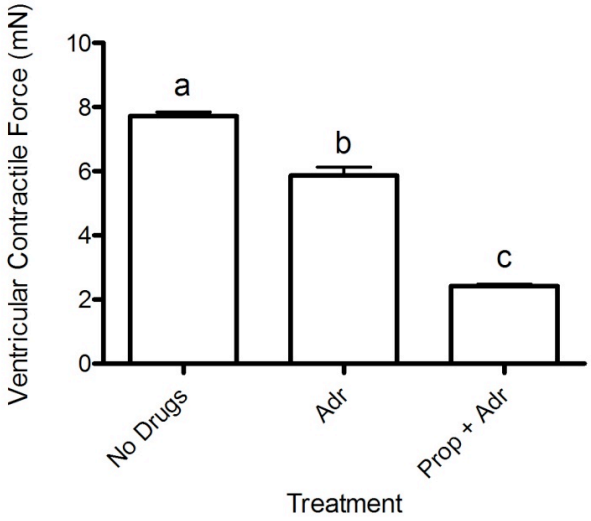

Figure 2: Mean + SEM ventricular contractile force (mN) of the cane toad (*Bufo marinus,* n=1*)* under treatment with no drugs, adrenaline (3 drops, 1mM), propranolol (3 drops, 1mM) and propranolol + adrenaline (3 drops each, 1mM). Different superscript letters denote significant differences at $p<0.05$.

DISCUSSION:

This experimental investigation has shown that drug treatment does have a significant effect on the heart rate and ventricular contractile force of a cane toad. The treatment of the toad heart with agonistic catecholamines, such as adrenaline, is thought to induce a signal transduction pathway which changes the influx of ions into the different cell types which possess adrenergic receptors (Ju and Allen, 1999 and DiFrancesco, 1993). Ultimately this ion influx regulates the release of sarcoplasmic reticulum calcium stores (Ju and Allen, 1999; O'Donnel and Wanstall, 1982). An increase in intracellular free calcium ion concentration is associated with the increase in both heart rate and contractile force and a decrease produces the opposite effect (O'Donnel and Wanstall, 1982; Allen and Blinks, 1978).

Considering the increase in heart rate observed in treatment with adrenaline, a potential explanation is that receptor activation of pacemaker cells allowed an influx of ions which enabled the sarcoplasmic release of Ca^{2+}. This coincides with Hancox et al.'s (1994) finding that β adrenergic receptor stimulation increases the rise of the systolic atrioventricular free calcium ion concentration. In addition to this the results of research by Ju and Allen (1999) which showed that Ca^{2+} release from the

sarcoplasmic reticulum in pacemaker cells is essential for spontaneous generation of autonomic action potentials. Consequently, it can be postulated that the stimulation of β adrenergic receptors by adrenaline in pacemaker cells of a Bufo *marinus* results in a signal transduction pathway in which calcium is released from the sarcoplasmic reticulum. The elevation of free calcium ion concentration in the cytosol is thought to then cause an increase in the frequency of action potential generation (O'Donnel and Wanstall, 1982).

Research by Eisner et al. (2000), Morris et al. (1981) and A. Ask (1983) has shown an increase in heart rate in the treatment with propranolol and adrenaline when compared to controls. This has been attributed to the non-blockaded sympathetic nerve stimulation (Morris et al., 1981; Bramich et al., 1990). However, this contradicts this investigation's results as there was a reduction in heart rate observed. Research by Messineo and Katz (1979) on mammalian β receptors has identified an inhibitory effect of propranolol on β adrenergic receptors. The antagonistic binding of receptors such as propranolol was shown to reduce the uptake of Ca^{2+} into the sarcoplasmic reticulum and this limits the magnitude of concentration change of free calcium ions (Messineo and Katz, 1979). Consequently, the heart rate is reduced (Ju and Allen, 1999). This negative inhibition of adrenergic receptors represents a potential mechanistic explanation of the reduction in heart rate for the combined propranolol and adrenaline treatment.

With regard to the ventricular contractile force under a treatment with adrenaline, the field research is variable in observed physiology. Lazou (2002) and Aggeli et al (2002) achieved results which supported an increase in contractile force via α_1 receptor stimulation. This is combined with the positive inotropic effect of both β receptor activation (O'Donnel and Wanstall, 1982) and unidentified sympathetic nerve stimulation (Morris et al., 1981). This research is contrary to the findings of this investigation. These results are supported by Li et al. (1997) who found that adrenaline acted to decrease ventricular contractile force. The mechanism for this was postulated as the increased myofibrillar affinity for Ca^{2+} (Li et al. 1997). Whilst myofibrillar binding of Ca^{2+} is attributed to activating contraction (Fuchs and Briggs, 1968), the increased affinity prevents relaxation of the muscle cells. Consequently, the cardiac muscle cells do not completely relax before re-stimulation and thus the force of contraction is reduced (Fuchs and Briggs, 1968). This difference between results and field research is possibly due to condition dependent behaviour of the adrenergic receptors such as the change in conformation hypothesised by Kunos and Nickerson (1976).

The results showed a further reduction in ventricular contractile force for treatment with propranolol and adrenaline when compared with the two alternative treatment categories. This may be due to the reduction of calcium uptake into the sarcoplasmic reticulum of the cardiac muscle cells as a result of propranolol binding (Messineo and Katz, 1979). This once again limits the calcium release which reduces the intracellular free calcium ion concentration thereby resulting in a reduction in ventricular contractile force (Allen and Blinks, 1978).

This study has produced results which differ from much of the present field research and this demonstrates the contentious nature of the scientific theory related to the Bufo *marinus* heart. It has been shown that drug treatment does have a significant effect on the heart rate and ventricular contractile force. The role of calcium as a regulator of cardiac function has been proposed as a biological reason for the variation between research experiments. Consequently, in order for the scope of knowledge in this field to develop, future experiments should focus on the specific changes in intracellular free calcium ion concentration when considering the observed physiological activity of cardiac cells.

REFERENCES:

Aggeli, I., Gaitanaki, C., Lazou, A., Beis, I., (2002), *α_1- and β-adrenoreceptor stimulation differentially activate p38-MAPK and atrial natriuretic peptide production in the perfused amphibian heart*, The Journal of Experimental Biology, Vol. 205, pp. 2387-2397

Allen, D., Blinks, J., (1978), *Calcium transients in aequorin-injected frog cardiac muscle*, Nature, volume 273, pp. 509-513

Ask, J., (1983), *Comparative aspects of adrenergic receptors in the hearts of lower vertebrates*, Comparative Biochemistry and Physiology Part A: Physiology, Vol. 36, pp. 543-552

Benfey, B., (1977), *Cardiac Adrenoreceptors at Low Temperature and the Adrenoreceptor Interconversion Hypothesis*, Journal of Pharmacology, Vol. 61, pp. 167-173

Bramich, N., Edwards, F., Hirst, G., (1990), *Sympathetic Nerve Stimulation and Applied Transmitters on the Sinus venosus of the Toad*, Journal of Physiology, Vol. 429, pp. 349-375

DiFrancesco, D., (1993), *Pacemaker mechanisms in cardiac tissue*, Annual Review of Physiology, Vol. 55, pp. 455-472

Eisner, D., Choi, H., Diaz, M., O'Neill, S., Trafford, A., (2000), *Integrative Analysis of Calcium Cycling in Cardiac Muscle*, Journal of the American Heart Association, Vol. 87, pp. 1087-1094

Fuchs, F., Briggs, N., (1968), *The Site of Calcium Binding in Relation to the Activation of Myofibrillar Contraction*, The Journal of General Physiology, Vol. 51, pp. 655-676

Hancox, J., Levi, A., Brooksby, P., (1994), *Intracellular calcium transients recorded with Fura-2 in spontaneously active myocytes isolated from the atrioventricular node of the rabbit heart.* Proceedings of the Royal Society of London B, Vol. 255, pp. 99-105

Ju, Y., and Allen, D., (2000), *The distribution of calcium in toad cardiac pacemaker cells during spontaneous firing*, European Journal of Physiology, Vol. 441, pp. 219-227

Kunos, G., Nickerson, M., (1976), *Temperature-induced interconversion of α- and β-adrenoreceptors in the frog heart*, Journal of Physiology, Vol. 163, pp. 484-504

Lazou, A., Markou, T., (2002), *Phosphorylation and activation of mitogen- and stress- activated protein kinase-1 in adult rat cardiac myocytes by G-protein-coupled receptor agonists requires both extracellular-signal-regulated kinase and p38 mitogen-activated protein kinase*, Biochemical Journal, Vol. 365, pp. 757-763

Li, K., He, H., Li, C., Sirois, P., Rouleau, J., (1997), *Myocardial α_1-adrenoreceptor: Inotropic effect and physiological and pathological implications*, Life Sciences, Vol. 60, pp. 1305-1318

Maxwell, S., and Webb, D., (2008), *Receptor functions*, Medicine, Vol. 36, pp. 344-349

Messineo, F., and Katz, A., (1979), *Effect of Propranolol and Timolol on Calcium Uptake by Sarcoplasmic Reticulum Vesicles*, Journal of Cardiovascular Pharmacology, Vol. 1, pp. 449-459

Morris, J., Gibbins, I., Clevers, J., (1981), *Resistance of Adrenergic Neurotransmission in the Toad Heart to Adrenoreceptor Blockade*, Naunyn-Schmiedeberg's Archives of Pharmacology, Vol. 317, pp. 331-338

O'Donnel, S., Wanstall, J., (1982), *Pharmacological Experiments Demonstrate that Toad (Bufo marinus) Atrial Beta-Adrenoreceptors are not Identical with Mammalian Beta 2 or Beta I – Adrenoreceptors*, Life Sciences, Vol. 31, pp. 701-708

Seraskeris, S., Lazou, A., (2002), *α_1-adrenergic stimulation mediates Ca^{2+}-dependent inositol phosphate formation through the α_{1B}-like adrenoreceptor subtype in adult rat cardiac myocytes*, Journal of Cellular Biochemistry, Vol. 84, pp. 201-210