Effects of adrenaline and propranolol on heart rate and contractility in *Bufo marinus*.

Introduction

The heart is an integral part of the cardiovascular system and is thus subject to sophisticated regulatory mechanisms originating from both its intrinsic properties as well as hormonal and neural signals (Boron,2009). One such regulatory mechanism is the sympathetic stimulated release of catecholamines, including adrenaline by the sympathetic nervous system (Boron, 2009). Adrenaline is thought to exert its primary influence through β adrenoreceptors in the mammalian heart (Lodish et.al, 2008). Amphibian hearts however, differ significantly from the mammalian and the receptors present have not been fully characterised (Buckley & Jordan, 1970).

Intrinsically, heart rate is determined by the action of pacemaker cells, located in the sinus venous region of the toad heart (Ju et.al, 1995). When the pacemaker cell is hyperpolarized a small inward positive current, which has not been fully typified in toads, is activated, slowly depolarising the membrane. When the membrane potential reaches threshold value around -50mV, the voltage sensitive L-type Ca^{2+} channels open, allowing Ca^{2+} influx and membrane depolarization (Ju et.al, 1995; Boron, 2009). This action potential is propagated to surrounding cells, including cardiac myocytes, via gap junctions (Boron & Boulpaep, 2009). Additionally, the influx of Ca^{2+} through the L-type calcium channels stimulates nearby ryanondie receptors (RyR) in the sarcoplasmic reticulum (SR) membrane which then release more Ca^{2+} into the cell, a process termed Calcium induced Calcium release (CICR)(Soeller & Cannell, 2004). This intracellular Ca^{2+} binds to the tropomyosin complex surrounding actin in muscle cells, causing myosin binding sites to be exposed and allowing cross bridge cycling to occur (Boron & Boulpaep, 2009). As repolarizatoin occurs, Ca^{2+} is pumped back into the sarcoplasmic reticulum and out of the cell returning the actin filaments to inactivated state (Boron & Boulpaep, 2009).

Exogenous application of adrenaline to the toad heart is believed to primarily activate β-adrenorecptors (Bramich et.al, 2001). Adrenaline binds to the β- adrenergic G protein coupled receptor, causing a conformational change which results in the $G_{\alpha s}$ protein subunit dissociating and activating adenylyl cyclase (Lodish et.al, 2008). This results in synthesis of the second messenger cAMP, which then activates a cAMP dependent protein kinase (Lodish et.al). This kinase is thought to phosporylate, thus modifying, various proteins involved in pacemaking and excitation contraction, making them more sensitive to a lower voltage or increasing their capacity to allow flux of ions through them (Bramich et.al, 2001).

Increased passive influx of ions will result in the threshold membrane potential being reached more rapidly. Additionally, if L-type Ca^{2+} channels are more sensitive, the depolarization threshold will be lower in cells which have been stimulated with adrenaline (Boron, 2009; Soeller & Cannell, 2004). The K^+ channel also allows greater efflux of K^+, leading to a more rapid repolarisation (Soeller & Cannell, 2004). These measures will all result in faster generation of action potentials and thus an increased heart rate. A larger influx of Ca^{2+} combined with the increased sensitivity of the RyR release channel results in a larger CICR (Boron & Boulpaep, 2009). The increased Ca^{2+} concentration combined with the increased sensitivity of the contractile apparatus to Ca^{2+} as a result of phosphorylation means that more binding sites will be exposed and increased cross bridge cycling will occur, leading to increased ventricular contractile force (Soeller &Cannell, 2004).

Propranolol is a β-adrenergic antagonist that competitively binds to the receptor. By occupying these binding sites propranalol can inhibit the actions of agonists, preventing receptor signalling

(Lodish et.al, 2001). Propranalol has been found to exhibit no effects on heat rate and Ca^{2+} concentration when applied in isolation but lead to a significant decrease in the potency of adrenaline (Ju & Allen, 1999). Thus if adrenaline acts primarily through B-adrenoreceptors, in the toad heart, the addition of propranolol should largely mitigate its effects

Hypothesis

The addition of adrenaline to the heart of a cane toad (*Bufo marinus*) will increase heart rate and ventricular contractile force as compared to basal heart activity, while the application of propranolol prior to the addition of adrenaline will result in basal heart rate and contractile force remaining unchanged.

Method

Subjects

The experiment was conducted on one double pithed cane toad (*B. marinus*). The destruction of the animal's nervous system ensured that it did not feel any pain and that the experimental results were not affected by hormonal responses from the animal. The toad was dissected longitudinally along the abdomen, with an additional lateral incision across the chest to open up the toad. The sternum and pericardium tissue were cut away to expose the heart. The heart was connected to a force transducer by inserting a pin through the apex of the ventricle. This pin was connected by a taut string to the transducer, suspending the heart outside the thoracic cavity. Frog ringers' solution was applied regularly throughout this procedure. An ECG was set up by attaching the negative lead to the right forelimb and the earthing lead to the right hind limb via alligator clips. The positive electrode was attached to a small wire which was inserted into the ventricle wall such that it did not come into contact with the pin of the force transducer.

Experimental Protocol

The ECG and force transducer readings were recorded using PowerLab software and displayed as a trace in LabChart. The basal electrical and mechanical activity of the heart was recorded for two minutes and then used as the negative control for the experiment. Three drops of 1mM adrenaline were applied to the toad heart using a 1.5mL pipette and the resulting cardiac activity recorded over two minutes. The heart was then repeatedly rinsed with frog ringer solution in order to remove the drug. Three drops of 1mM propranolol was then added to the heart with a separate 1.5mL pipette and 30 seconds later three drops of adrenaline were added. Mechanical and electrical activity was recorded throughout this procedure. After an additional three minutes the heart was washed with Frog Ringer's in order to remove the drugs. This protocol was repeated three times.

Data Analysis

For each treatment condition, the mean heart rate was found by counting the number of ECG peaks in 30 seconds in each of the three trials and averaging this result to obtain heart rate in beats per minute. Mean ventricular contractile force was obtained using LabChart to find the difference between the maximum and minimum values of contractile force in a single heart beat for each condition in each of the three trials. The mean and standard error of the mean were calculated for the force of ventricular contraction and the heart rate. A one-way ANOVA with Tukey's post test was used to determine the effect of adrenaline by itself and adrenaline and propranolol together on heart rate and also ventricular contractile force. Statistical significance will be accepted at $P< 0.05$.

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Results

There was a significant decrease in heart rate during treatment with adrenaline & propranolol compared to control (P< 0.05, Figure 1). Adrenaline by itself did not exert a significant difference on heart rate. There were no significant differences between treatments for contractile force.

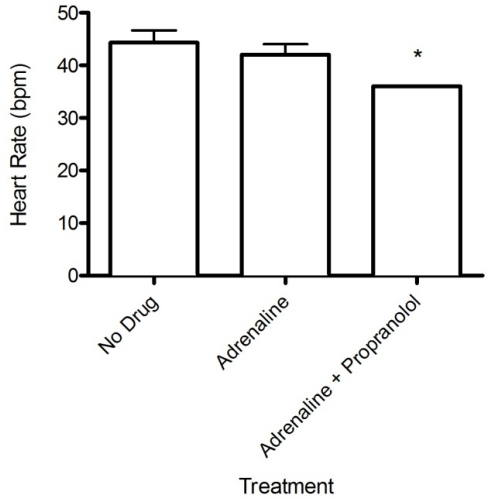

Figure 1: Mean±SEM of heart rate (bpm) of the cane toad (Bufo Marinus, n=1) under treatment with no drugs, adrenaline (3drops, 1mM) and propranolol + adrenaline (3 drops, 1mM, each). * indicates that treatment with adrenaline + propranolol leads to a lower heart rate then the control (P<0.05)

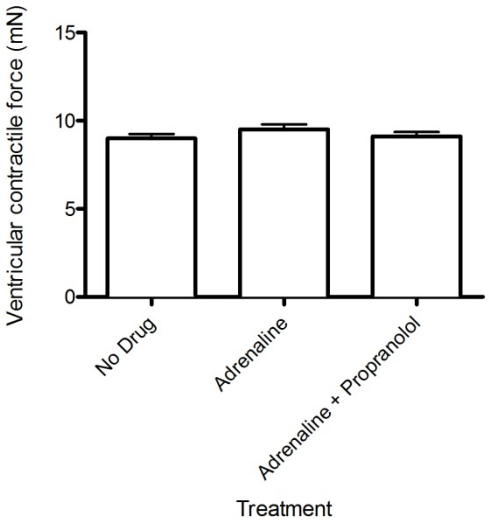

Figure 2. Mean ± SEM of ventricular contractile force (mN) of the cane toad (Bufo Marinus, n=1) under treatment with no drugs, adrenaline (3drops, 1mM) and propranolol + adrenaline (3 drops, 1mM, each).

Discussion

This experiment found that the exogenous application of 1mM adrenaline had no effect on heart rate or contractile force in the toad heart (*B. marinus*). This was inconsistent with the hypothesis that adrenaline would up regulate both these aspects of cardiac function. Previous experimental studies of the toad heart also contradict these results as they found significant increases in heart rate and contractility in the presence of adrenaline (Yue-Kun & Allen,2006; Kunos & Nickerson, 1975). The most surprising result obtained was the decrease in heart rate in the presence of propranolol. It was hypothesised that the addition of propranolol would maintain basal heart rate as it is believed to be a β-blocker, not a signalling molecule.

Ju and Allen (2006) found that β-adrenergic stimulation with exogenous adrenaline significantly increased the amplitude of the internal Ca^{2+} transient as well as the increasing the firing rate of pacemaker cells in *B.marinus* by 50%. They also determined that the relationship between the Ca^{2+} transient amplitude and firing rate was the same regardless of whether the increased entry of Ca^{2+} to the cell was caused by β -adrenergenic stimulation or not (Ju & Allen, 2006). This suggests that it is the increased transient calcium concentration that is directly responsible for the more rapid action of pacemaker currents in toad hearts rather than a change in the intrinsic properties of the ion channels themselves (Ju & Allen, 2006). In particular, it has been suggested that the Na^{+} Ca^{2+} exchanger might play an important role in coupling increased calcium transients to faster pace in amphibians as it relies on differences in Ca^{2+} concentrations to drive its functions and can cause depolarization at negative membrane potentials (Ju & Allen, 2006; Blaustein & Lederer, 1999).

Similarly, Bramich et. al (2000) found that treatment of the pacemaker region of the toad with adrenaline caused an increase in the maximal values of the transient internal Ca^{2+} concentration and faster heart rate through the cAMP dependent pathway and activation of phosporylating kinases. Activation by adrenaline of pathways that increase influx of Ca^{2+} through the L-type Ca^{2+} channels and sarcoplasmic reticulum were clearly non operational in this experiment.

The rise intracellular Ca^{2+} such as that detected in previous studies of the toad heart has been intimately linked with increased crossbridge cycling (Boron & Boulpaep, 2009). This indicates that in the cardiac myocytes of toads, greater contractile force should be observed in the presence of adrenaline. Morris et.al (1981) found that treatment with adrenaline increased the force of atrial contraction by 37.5% from basal rate - an increase that was abolished by subsequent treatment with propranolol. However, no increase in contractile force was observed in this experiment.

Receptor pathways which increase the transient calcium current, leading to increased contraction and pacemaker activity may have been inhibited by temperature. The heart was suspended outside the thoracic cavity, in a section of the laboratory that was significantly below 25°C. Kunos and Nickerson (1975) assert that temperature changes the properties of the adrenoreceptors in frog hearts. They found that at cold temperautes, the presence of α-adrenoreceptors became more common and any chemicals acting primarily through β-receptors had a diminished effect. Similar observations on the importance of temperature were made by Buckley and Jordan (1970) who showed that the effects of adrenaline were abolished at 7°C. This phenomenon may have contributed to the maintenance of basal heart rate despite the presence of adrenaline.

In addition to finding no significant change with the addition of adrenaline, the combination of adrenaline and propranolol was found to significantly decrease heart rate compared to basal levels in contradiction with most current literature. Propranolol is widely accepted to be a competitive ligand which exerts no independent effects (Lodish et.al, 2001). Separate studies have found that after propranolol was added to a heart stimulated by adrenaline, basal levels of action potential frequency were restored, showing no significant difference with the control (Ju & Allen, 2006; Kunos & Nickerson, 1975). However, there is some evidence that the effects of propranolol may go beyond B-adrenergic receptor blockage.

Morris and Gibbons (1981) used hyoscine drug to prevent an inhibitory parasympathetic response and phentolamine to block alpha receptors . Electrical stimulation of sympathetic nerves lead to an increase in the force of atrial beat of 110.5% and the heart rate increased by 32%. These values remained elevated for several minutes before returning to approximately pre-stimulation levels which is consistent with the release of adrenaline from the sympathetic nervous system. However, when propranolol was present and stimulation was stopped, heart rate and force were seen to continue to decline below pre-stimulation levels for 60 – 90s (Morris & Gibbons, 1981). Although this result provides little insight into the molecular mechanism as the presence of other drugs may have had a significant effect, this finding suggests that the actions of propranolol may go beyond simply acting as a competitive inhibitor and in some situations actively decrease heart rate. Further investigation into this phenomenon and the types of receptors which mediate the response of the toad heart to adrenaline (both exogenously and sympathetically supplied) and propranolol is necessary to explain the observed decrease in heart rate in this experiment and lack of change in other treatment groups.

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