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The effects of ethanolamine and magnesium on cardiac and neurological function in isoprenaline-induced myocardial infarction and cardiac hypertrophy models in adult Wistar rats.

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GRSCHR003

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"The will of God will never lead you where the grace of God cannot keep you"

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LIST OF ABBREVIATIONS

µL – Microliter

µm – Micrometer

µMol – Micromolar

•OH – Hydroxyl Radicals

ADP – Adenosine Diphosphate

AMP – Adenosine Monophosphate

Ang II – Angiotensin II

ARIC – Atherosclerosis Risk in Communities

ATP – Adenosine Triphosphate

Avg - Average

BP – Blood Pressure

bpm – beats per minute

BW – Body Weight

Ca^{2+} - Calcium

CAD – Coronary Artery Disease

CD – Conjugated Dienes

CVD – Cardiovascular Disease

DNA – Deoxyribonucleic Acid

dP/dt min – minimum rate of left ventricular pressure decline

dP/dt max – maximum rate of left ventricular contraction

ECG – Electrocardiogram

EPM – Elevated Plus Maze

Etn – Ethanolamine

FAAH – Fatty Acid Amide Hydrolase

FST – Forced Swim Test

g – Gravity

H&E – Haematoxylin and Eosin

H2o2 – Hydrogen Peroxide

HF – Heart Failure

HPA – Hypothalamic-Pituitary-Adrenal

HW/BW – Heart Weight to Body Weight

ISO – Isoprenaline

I_{to} – Transient Outward Current

IU – International Units

JAK – Janus Kinase

K+ - Potassium

Kg – Kilogram

L – Liter

LDH – Lactate Dehydrogenase

LVEDP – Left Ventricular End Diastolic Pressure

M – Molar

mg – milligram

Mg^{2+} - Magnesium

MI – Myocardial Infarction

Min – Minute

ml – Milliliter

mM – milliMolar

mmHg – millimetres of Mercury

mmol – milliMoles

MMPs – Matrix Metalloproteinases

mPTP – Mitochondrial Permeability Transition Pore

mRNA – messenger Ribonucleic Acid

ms – millisecond

mV – milliVolts

Na^{+} - Sodium

nM – nanoMolar

O2•- - Superoxide Anion Radical

OCT – Optimal Cutting Temperature

OF – Open Field

PRWP – Poor R-Wave Progression

QTc – QT corrected for heart rate

ROS - Reactive Oxygen Species

s – seconds

S1P – Sphingosine-1-phosphate

SAFE – Survival Activating Factor Enhancement

SEM – Standard Error of the Mean

SSA – Sub-Saharan Africa

STAT-3 – Signal Transducer and Activator of Transcription 3

TBA – Thiobarbituric acid

TBARS – Thiobarbituric Acid Reactive Substances

TNF-α – Tumour Necrosis Factor

TRP – Transient Receptor Potential

TRPM 6 – Transient Receptor Potential Melastatin 6

TRPM 7 – Transient Receptor Potential Melastatin 7

TTC – Triphenyltetrazolium Chloride

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ABSTRACT

Background: Myocardial infarction (MI) is a principal cause of cardiovascular morbidity and mortality that is associated with other systemic complications. In the heart, MI can result in pump dysfunction, inducing cardiac hypertrophy which may become maladaptive leading to heart failure (HF). In the brain, MI is associated with psychological disorders such as anxiety and depression. Many pharmacological agents have been identified to modulate MI and hypertrophy development. The effects of one such agent, magnesium (Mg^{2+}), often used in the treatment of cardiac arrhythmias and hypertension, was further explored as MI pre-treatment. Also, the effects of a novel agent, ethanolamine (Etn), previously been shown to be cardioprotective *ex vivo*, were assessed *in vivo*. Etn deficiency has also been linked to the occurance of neurological dysfunction, yet its effects on MI-related neurological disturbances remain elusive. Both Mg^{2+} and Etn are readily available in the African diet and therefore may provide a cost-effective therapy against MI.

Methods: Male Wistar rats (250-300g) were used to study MI and hypertrophy. For both pathologies, isoprenaline (ISO) was used to induce cardiac stress (MI model: 67 mg/kg s.c. single injection; hypertrophy model: 5 mg/kg i.p. for seven consecutive days). The effects of Etn were investigated in both MI (n=46) and hypertrophy models (n=29) while Mg^{2+} was tested in the MI model (n=35). All control rats received equivalent volumes of saline. Behavioural tests for anxiety and depression were conducted two hours prior to anaesthesia before invasive *in vivo* assessment, using the elevated plus maze (EPM), open field (OF) and forced swim (FST) tests. Rats were anaesthetised 24 hours after the last ISO injection. After anaesthesia, rats were intubated and ventilated, and the carotid artery was cannulated. Electrocardiogram (ECG), carotid arterial and left ventricular blood pressure (BP) were recorded online via the PowerLab data-acquisition system, before the heart and other organs were extracted. Products of lipid peroxidation, conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) in plasma were measured by spectrophotometry. Infarct size was measured using triphenyltetrazolium chloride (TTC) staining. Hypertrophic changes were evaluated using haematoxylin and eosin (H&E) staining of myocardial cryosections.

Results: In the MI model, ISO produced a significantly larger TTC-negative, infarcted myocardial area compared to controls (64 ±3% vs. 24 ±2%; P<0.001) and increased the heart weight to body weight (HW/BW) ratio (P<0.001). ISO decreased systolic and diastolic BPs, without changing heart rate and induced a low-voltage ECG with pathological Q-waves. Systemically, ISO caused a loss in body weight (BW) and decreased the liver/BW ratio. Etn did not alter ISO-induced infarct size, but decreased the number of ISO-related deaths six-fold. Etn also caused further increase in HW/BW ratio (5.0 ±0.2 vs. 4.6 ±0.1, P<0.05, compared to ISO-treated rats), further increased loss in BW (P<0.001 vs. control) and prevented the ISO-induced decrease in the lungs/BW ratio. Mg^{2+} improved on ISO-induced changes in haemodynamic parameters but caused a further loss in BW. In the hypertrophy model, ISO caused fibrosis and necrosis to the myocardium visible on H&E stain. Etn again amplified hypertrophy compared to ISO-treated rats (HW/BW ratio = 5.5 ±0.2 vs. 5.2 ±0.1, P<0.05) and improved various ECG parameters. Etn also lowered the heart rate compared to control rats (389.1 ±11.7 vs. 324.5 ±9.5, P<0.01). The behaviour of the rats was unchanged by ISO or Etn treatment.

Conclusion: Etn decreased ISO-related mortality, possibly via augmentation of compensatory hypertrophy. Mg^{2+} pre-treatment reduced ISO-induced hypotension but did not minimise infarction therefore whether Mg^{2+} should remain a drug of choice in treating MI patients needs further consideration. In a model of hypertrophy Etn also amplified the hypertrophic response and reduced heart rate. Further investigations are required to assess Etn's impacts on neurological function.

INTRODUCTION

Myocardial infarction (MI) is a complication of cardiovascular disease (CVD) that is usually associated with elderly people in high and middle-income countries due to the urbanised lifestyle (Longo et al., 2012). Due to the increasing control of communicable diseases and the evolving Westernised societal shift in developing countries, the prevalence of MI is increasing in Africa (Yusuf et al., 2001; Mendez and Cowie, 2001; White and Chew, 2008). Recently, Mayosi et al. (2009) identified that cardiovascular complications are the second most common cause of death in men and women above the age of 65 years living in South Africa. Cardiomyocyte hypertrophy often occurs after MI and is initially an adaptive response to help compensate for the increased external load caused by the infarct and maintain the ejection fraction. However, with time the myocardial architectural alteration becomes maladaptive and the patient may develop left ventricular dysfunction that culminates in heart failure (HF) and mortality (Erlebacher et al., 1984; Pfeffer and Braunwald, 1990). Novel therapies are required to limit the insult caused by the initial MI and to minimise or prevent the development of pathological hypertrophy.

To assess the impact of novel therapies, an animal model of MI and pathological cardiac hypertrophy must be defined. There are various methods used to induce infarction in animals such as coronary artery ligation, cauterisation and an overdose of catecholamines such as isoprenaline (ISO). ISO, a synthetic catecholamine and an analogue of epinephrine (Rona et al., 1959; Nirmala and Puvanakrishnan, 1994), is a β-adrenergic receptor agonist that, when administered acutely in high doses, results in infarct-like lesions similar to those found during human MI (Rona et al., 1959). However, a survey of the literature indicates that ISO-induced MI is associated with a high mortality because of large doses injected too frequently (Wexler, 1979; Judd et al., 1969; Crandall et al., 1981). There is a need for a model of MI that provides a low mortality rate but also a measurable outcome (eg. infarct size). In the studies described in this thesis, such a model was established and after characterisation, the effects of novel therapies on MI development were investigated using the model.

Pharmacological agents have long been used to treat MI and this thesis examines the cardioprotective effects of a novel agent, ethanolamine (Etn), and of a long-standing therapeutic agent, magnesium (Mg^{2+}) in the pre-treatment of ISO-induced MI. Etn is a biogenic amine, found exogenously in food and drinks and is also present endogenously in the body (Bitman et al., 1984; Pfeiffer and Radler, 1992; Caruso et al., 2002). Etn has previously been shown to protect the isolated rat heart from ischaemia-reperfusion injury (Kelly et al., 2010) however; its effects *in vivo* are unknown.

The second therapeutic agent studied was Mg^{2+}. Mg^{2+} is a divalent cation found abundantly in vegetables and legumes. In a review paper, Elin (1994) stated that Mg^{2+} is often the "forgotten" electrolyte in medical research, even though it is involved in over 300 enzymatic reactions. Currently, Mg^{2+} is often used in the treatment of arrhythmias and hypertension (Seller et al., 1970; Watson et al., 1986). The effects of Mg^{2+} therapy pre-MI have not been thoroughly researched and there are controversies as to the use of Mg^{2+} as a cardioprotective agent (The Second Leicester Intravenous Magnesium Intervention Trial and The Fourth International Study of Infarct Survival). An investigation is required to assess whether Mg^{2+} administered pre-MI reduces complications post-MI.

Chronic ISO administration provides a model for the development of pathological cardiac hypertrophy (Meszaros, 1992; Inamoto et al., 2000; Ennis et al., 2003; Hanada et al., 2008). Etn pre-treatment has not yet been investigated in a model of cardiac hypertrophy, therefore the effects of Etn on the development and progression of cardiac hypertrophy was investigated.

There is a link between cardiac dysfunction and neurological complications such as anxiety and depression (Prickaerts et al., 1996; Grippo et al., 2003; Wann et al., 2007; Rousseau et al., 2012). The prevalence of anxiety and depression, post-MI, impacts on morbidity and mortality (Frasure-Smith et al., 1995; Carney et al., 2004; Larsen et al., 2010). Although not directly, Etn has been implicated to impact beneficially on neurological deficits (Nitsch et al., 1992; Matas et al., 2007) but further research is required to assess whether Etn can protect neurological function after MI.

REVIEW OF LITERATURE

2.1 Myocardial Infarction and the Burden of Cardiovascular Disease

MI is a complication of many CVD and contributes to high mortality rates. With advancements in the control of communicable diseases and malnutrition, the World Health Organisation predicts that by 2020, CVD will have reached a state of global epidemic (Muna, 1993; Mendez and Cowie, 2001). In North America, which is a predominantly western society, CVDs account for 50% of deaths whereas CVD accounts for only 25% of deaths in developing countries (Mendez and Cowie, 2001). Even so, the prevalence of MI is growing rapidly in developing countries and it is predicted that the mortality rate from ischaemic heart disease in the year 2020 will increase by 120% for women and 137% for men (Steyn et al., 2005).

Cardiomyocyte hypertrophy will often occur after MI and is considered to initially be an adaptive response to help compensate for the increased external load that the infarct has caused and to maintain the ejection fraction (Pfeffer and Braunwald, 1990; Richey and Brown, 2001). The hypertrophy eventually becomes maladaptive and the patient develops HF (Rouleau et al., 1993). HF is accountable for approximately 25% of all deaths in developing countries and patients suffering with HF have a 50% mortality rate within 4 years (Lopez, 1993; Dickstein et al., 2008). Therefore Gaudron et al. (1993) state that multifactorial interventions are required to restrict the development of pathological hypertrophy before dilatation of the left ventricle has even occurred. The sequelae of MI are broad and include cardiovascular manifestations and complications to other sites such as the nervous system. MI is associated with neurological problems for example depression and anxiety. In turn, the emotional stress and subsequent activation of the hypothalamic-pituitary-adrenal (HPA) axis as well as the sympathoadrenal system can trigger the occurrence of a severe cardiomyopathy (Ueyama et al., 2008).

2.1.1 The Risk of Cardiovascular Disease in South Africa

The increasing mortality rates from CVD in developing countries, specifically Sub-Saharan Africa (SSA), could be due to an increased life expectancy allowing patients to become

susceptible to CVDs (Hasenfuss, 1998; Cowie et al., 1997). Poor health care systems, improved management of malnutrition and infectious diseases, urbanisation and an increase in cardiomyopathies associated with HIV/AIDS and HIV treatment may also be factors (Herskowitz et al., 1992; Barbaro, 2003; White and Chew, 2008). Furthermore there are advancements in the understanding and clinical management of ischaemic heart disease, allowing people to live longer with the condition (Hasenfuss, 1998). Despite these factors, currently in SSA, 7-10% of medical admissions are related to CVD (Damasceno et al., 2007). In South Africa, cardiovascular complications accounts for 9% and 11% of deaths in men and women over 65 years respectively (Mayosi et al., 2009). This is posing a burden to the region and its economy through resource depletion and inefficiencies of the workforce (Damasceno et al., 2007; Cowie et al., 1997).

South Africa is shifting towards a more urban lifestyle as it is one of the wealthiest nations in Africa. The risk of MI increases with urbanisation, which may be attributed to elevations in obesity and hypertension (Yusuf et al., 2001; White and Chew, 2008). As hypertension causes an increase in cardiac afterload, the demand placed on the ventricles will be greater to maintain the ejection fraction, thus the patient may develop HF. Urbanisation can result in the emergence of Type A personality traits (impatience, hostility, competitiveness, restlessness) which can cause disorders such as stress, anxiety and hypertension, all of which are risk factors for CVD (Singh et al., 2002). An alteration of eating habits and a decrease in exercise routines during urbanisation, for economic and convenience purposes, can lead to the accumulation of atherosclerotic plaques (Yusuf et al., 2001, Damasceno et al., 2007). The plaques are formed by excess low-density lipoproteins in the diet and can result in MI due to the reduction in blood flow to the myocardium (Fuster et al., 1994). Obesity is also becoming prevalent in SSA because of the stereotype associated with the HIV/AIDS epidemic, that underweight individuals are sick (Damasceno et al., 2007).

2.2 Myocardial Infarction

In a review by Opie and Swynghedauw (1991), it was suggested that the human heart can tolerate a reduced flow (ischaemia) of up to 20% of normal blood flow for approximately 30-45 mins. After this time, the ischaemia becomes fatal. This may be due to a critical loss of

adenosine 5'-triphosphate (ATP) causing sodium (Na^+)/potassium (K^+) pump dysfunction and disturbing the resting membrane potential (Cross et al., 1995). Disturbances in the resting membrane potential will decrease cardiac output and BP resulting in arrhythmias and MI (Jennings et al., 1986). There is also an overload of intracellular calcium (Ca^{2+}) during ischaemia causing membrane damage by inducing cell swelling (Marks, 2003). When this occurs in the mitochondria, the mitochondrial permeability transition pore (mPTP) is opened which activates apoptosis through swelling of the mitochondria due to the influx of water as well as uncoupling of oxidative phosphorylation (Halestrap, 2006). The outer mitochondrial membrane may rupture releasing pro-apoptotic proteins such as cytochrome complex, small mitochondria-derived activator of caspases and apoptosis-inducing factor (Gustaffson and Gottlieb, 2003). Remondino et al. (2003) add that during ischaemia apoptosis may occur due to the formation of free radicals. The free radicals also damage the myocardial cell membrane.

Opie and Swynghedauw (1991) further went on to address that another consequence of ischaemia is the poor washout of metabolites due to the decreased blood flow to the myocardium. The temporary anaerobic activity of the heart, due to the poor supply of oxygen, causes lactate to be produced from pyruvate and the hydrolysis of ATP. Anaerobic metabolism results in cellular acidosis through release of an inorganic phosphate from ATP which liberates the hydrogen ions found in the cytosol of the myocytes. The acidosis can also induce subsequent lysosomal activation leading to proteolysis and infarction. The accumulation of fatty acid metabolites due to a poor washout results in the breakdown of the phospholipid bilayer membrane (Guth et al., 1987; Opie and Swynghedauw, 1991).

2.2.1 Cell Death during Myocardial Infarction

Ischaemia initiates a series of pathophysiological events that arise due to the imbalance of oxygen supply and demand; these biochemical alterations have been termed the ischaemic cascade which initiates cell death (Nesto and Kowalchuk, 1987). Cell death commences within 15 to 40 mins after the cessation of blood supply to the heart (Hearse, 1990). After six hours, the majority of cells in the ischaemic region would have died. According to the wavefront phenomenon, cells in the endocardium are compromised first, as this is the area

of the heart where the greatest energy demand is, and epicardial cells die last (Reimer et al., 1977). A review by Edinger and Thompson (2004) summarised that cell death occurs via necrosis or apoptosis. Necrosis, which is deemed "passive cell death" occurs due to ATP depletion and is associated with cell membrane breakdown and inflammation. Apoptosis refers to "programmed cell death" in which there is fragmentation of chromosomal deoxyribonucleic acid (DNA) into apoptotic bodies, without membrane disturbance or inflammation. The apoptotic bodies are removed by phagocytosis which requires energy. There also may possibly be a third mechanism of death which is poorly understood, authophagy. Autophagy is the intracellular degradation of long-lived proteins and organelles, which has recently been evoked as a survival mechanism during myocardial stress (Gozuacik and Kimchi, 2004). The stimulus for autophagy is similar to that for necrosis and as such the two often occur in parallel (Edinger and Thompson, 2004).

2.2.1.1 Necrosis

Various factors have been proposed to induce necrosis in myocytes; some of which include Ca^{2+} overload, fatty acid metabolites, osmotic stress and free radical production. During reperfusion, Ca^{2+} accumulating in the mitochondria causes contraction band necrosis, an energy-consuming reaction which hypercontracts myocytes, tearing the cardiac myocytes apart from each other (Epstein et al., 1986). During ischemia, long chain fatty acids and their metabolites can alter the structure and the function of the membrane causing necrosis of myocardium (Katz, 1982). The overload of small molecules in the cytosol from ATP hydrolysis (adenosine diphosphate and inorganic phosphate), the breakdown of phosphocreatine (creatine and an inorganic phosphate) and glycogenolysis (glucose-1-phosphate) can result in plasma membrane rupture culminating in myocardial necrosis (Jennings and Steenbergen, 1985). Due to the lack of oxygen delivery in an ischaemic heart, the electrons are unable to combine with oxygen to form water, creating reactive oxygen species (ROS) which cause cell necrosis (Bolli, 1998). The hydrolysis of ATP also produces free radicals. Adenylyl cyclase converts adenosine diphosphate (ADP) to ATP and adenosine adenosine monophosphate (AMP). The dephosphorylation of AMP results in the formation of adenosine. Inosine is formed through the deamination of adenosine. Free radicals are produced when inosine is converted to xanthine (Hess et al., 1984; Hammond and Hess,

1985). It has also been noted that pro-inflammatory cytokines, which are attracted to damaged regions of the myocardium, may also be involved in free radical production (Ward et al., 1988).

2.2.1.2 Apoptosis

Myocardial cells that are subjected to severe ischaemia or reperfusion may experience apoptosis. This cell suicide is considered vital for organogenesis and tissue homeostasis (Lockshin and Williams, 1965). Apoptosis is a process of programmed cell death and can be characterised by cell shrinking, DNA fragmentation by endogenous endonucleases or fragmentation of the cell body (Kerr and Currie, 1972; Arends et al., 1990). The fragments are then phagocytosed (Kung et al., 2011). The damaged mitochondria release cytochrome C into the cytosol. This causes apoptosis via caspase activation (Antignani and Youle, 2006). It is accepted that apoptosis causes death of cardiomyocytes after ischaemia/reperfusion injury however whether apoptosis occurs due to ischaemia alone or in a combination with reperfusion has yet to be determined (Fliss and Gattinger, 1996; Olivetti et al., 1996; Saraste et al., 1997; Anversa et al., 1998; Toyoda et al., 1998; Piro et al., 2000; Zhao et al., 2000; Palojoki et al., 2001). There is also contention between animal and human studies regarding the area of the heart affected by apoptosis. Animal studies tend to show apoptosis is prevalent both in the ischaemic border zone and the ischaemic region; however, human studies isolate apoptosis to affecting cells only in the ischaemic border zone (Fliss and Gattinger, 1996; Olivetti et al., 1996; Saraste et al., 1997; Toyoda et al., 1998; Piro et al., 2000; Palojoki et al., 2001). Recently, Kung et al. (2011) argued that a large portion of necrosis that occurs after MI is regulated, thereby opening up the possibility of targeting "programmed necrosis" for pharmacological interventions for the treatment of MI and HF.

2.3 Cardiac Hypertrophy

Cardiac hypertrophy may occur in response to physiological growth stimuli in which the hypertrophy is beneficial; or pathophysiological stimuli (such as after MI) in which the hypertrophy is ultimately detrimental (Zak, 1984). Hypertrophic remodelling of the left ventricular cardiomyocytes (size, ability and shape) is regulated by gene expression, neurohormonal secretion and mechanical alterations (Pfeffer and Braunwald, 1990; Rouleau

et al., 1993). Initially the hypertrophy occurring after MI is an adaptive response to maintain the ejection fraction by compensating for the increased external load caused by the infarct. However eventually this hypertrophy becomes maladaptive and the patient may develop HF. Figure 1 illustrates three types of ventricular remodelling: concentric, eccentric or left ventricular dilatation. Pressure overload causes the myocyte to thicken and concentric hypertrophy of the left ventricle occurs (dotted line represents the direction of growth of the myocytes). When there is a volume overload, the myocytes lengthen causing ventricular dilatation and eccentric hypertrophy. If there is an infarct (shown in the last image), the mixed loading on the heart will cause both concentric left ventricular hypertrophy to compensate for the failing tissue and also dilatation of the left ventricle (dotted lines represent future, pathological growth direction of cardiomyocytes). Post-infarction there can be early remodelling (expansion of infarct area) and late remodelling (left ventricular dilatation) (Erlebacher et al., 1984; Sutton and Sharpe, 2000).

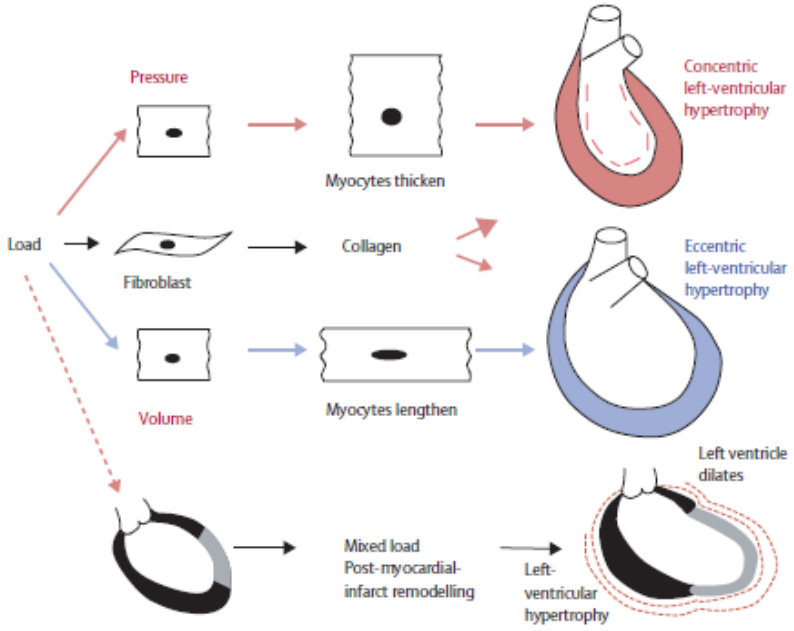


Figure 1: Pathophysiology of ventricular remodelling (Opie et al., 2006) adapted from Opie (2004). Figure reproduced with permission from the author.

2.3.1 Early Remodelling

Within hours of damage to the myocardium, neutrophils release matrix metalloproteinases which are activated in the cell causing an expansion of the infarct (Warren et al., 1988; Cleutjens et al., 1995). Serine proteases also degrade the intermyocyte collagen struts which

increases infarct size. Infarct expansion increases wall stress, which is regulated by mechanoreceptors involved in intracellular signalling via angiotensin II (Ang II) release. The release of Ang II increases the production of contractile units in the heart (Sadoshima et al., 1992). During this phase, the stroke volume is preserved by compensation of the non-infarcted zone. This alters the Frank/Starling relationship, increasing heart rate and shortening of the contractile elements which trigger the sympathetic adrenergic system to synthesize catecholamines (Lew et al., 1985). This activates the renin-angiotensin-aldosterone system to increase atrial and brain natriuretic peptides (Hall, 1996). This culminates in intravascular volume decrease and less systemic vascular resistance. There will be standard filling of the ventricles and pump function will be enhanced (Sutton and Sharpe, 2000).

2.3.2 Late Remodelling (Biochemical)

When catecholamines (such as norepinephrine) are released in early remodelling, stimulation of α1 and β1 adrenoreceptors occurs resulting in myocyte hypertrophy through signalling of the Gαq-dependant pathway and rennin release. This enhances the manufacturing of Ang II (Ball, 1989; Ju et al., 1998). Secretion of Ang II causes an increase of catecholamine synthesis, promotion of presynaptic release of norepinephrine, blocks reuptake of norepinephrine and increases endothelin-1 release. This stimulates hypertrophy of the non-infarcted region (Lindpaintner et al., 1993; Epstein et al., 1998). The remodelling process is also enhanced by the production of free oxygen radicals as oxidative stress is associated with apoptosis, fetal gene expression and myocardial hypertrophy given the involvement with protein synthesis and proliferation of fibroblasts (Murrell et al., 1990; Cheng et al., 1995; Colucci et al., 1997).

2.3.4 Late Remodelling (Mechanical)

An increase in the wall stress will activate the angiotensin receptor, AT1, to increase the secretion of Ang II (Sadoshima et al., 1992; Yamazaki et al., 1995). When this receptor is stimulated multiple signalling pathways are activated such as the tyrosine kinase, protein kinase C, mitogen-activated protein kinase and S6 kinase pathways (Ju et al., 1998). Mechanical stress will also activate early genes and the fetal gene program to induce

remodelling (Sadoshima et al., 1992; Yamazaki et al., 1995). Various stimuli to the myocardium, such as mechanical overload or neurohormonal activation, results in re-expression of the fetal genes in a compensatory attempt to keep up with the demand placed on the tissue. Synthesis of fetal proteins causes rapid hypertrophy of the myocytes, yet the myocardial quality and performance is poor affecting the diastolic function of the ventricles. This is the primary feature of remodelling that causes HF (Colucci, 1997; Swynghedauw, 1999). Systolic dysfunction also develops because there is no corresponding blood vessel growth (impairing the delivery of nutrients and oxygen) with increasing myocyte mass (Willenheimer, 2000). Remodelled cardiomyocytes also possess less mitochondria (impairing energy supply), less sarcoplasmic reticulum, Ca^{2+} channels and Ca^{2+} pumps all of which contribute to the failing of the ventricles (Katz, 1990; Swynghedauw, 1999).

2.4 Cardioprotection

As the global burden of disease is increasing the need for novel cardioprotective agents is growing. Pharmacological drugs are used to target MI prevention as well as the negative outcomes associated with MI. A recent review by Gerczuk et al. (2012) titled "An update on cardioprotection", identified that while the research conducted around pharmaceutical agents is promising, further investigation of novel drugs is required as well as confirmation of widely used drugs. The current study examined both a novel cardioprotective agent, Etn, and a long-used cardioprotective agent, Mg^{2+}.

2.4.1 Ethanolamine

Etn is a biogenic amine that is found endogenously in cell membrane lipids and as a metabolite of sphingosine-1-phosphate (S1P) and anandamide (Davitz et al., 1986; Matas et al., 2007). Both anandamide and S1P have previously been shown to be cardioprotective (Underdown et al., 2005; Jin et al., 2002). Etn is considered both an amine and an alcohol (Fig. 2). Etn is stored in the lipid membranes as phosphatidylethanolamine and exogenous Etn that is injected will alter the composition of phosphatidylethanolamine (Kano-Sueoka and Errick, 1981; Murakami et al., 1982). Exogenous Etn can be sourced exogenously from milk, wine and grapes (Bitman et al., 1984; Pfeiffer and Radler, 1992; Caruso et al., 2002).

The amount of Etn found in human breast milk is approximately 135.9µmol/L (Elmastas et al., 2008). Milk has recently been shown to be cardioprotective via its production of nitric oxide (Jirillo et al., 2010). Yet still little is known about the pathways of cardioprotection by milk and the potential influence of Etn. Wine itself is deemed to be cardioprotective through the actions of polyphenols and amines altering the concentrations of lipids in the body, as well as changing haemostatic parameters (Rimm et al., 1999; Das et al., 1999). Bacteria in wine degrade nitrogenous compounds which forms Etn (Bast, 1971). Etn is one of the amines found in both white and red wines (Buteau et al., 1984; Pfeiffer and Radler, 1992; Hlabangana et al., 2006; Hernandez-Borges et al., 2007). The concentration of Etn in wine varies from approximately 4 to 17 mg/L (Pfeiffer and Radler, 1992) (Table 1). Whether the cardioprotection associated with wine is dependant on the actions of Etn requires further exploration. The cardioprotection exhibited by grapes is generally thought to come from the proanthocyanidins (found in the grape seed and skin) ability to scavenge ROS which are produced during ischaemia-reperfusion injury (Sato et al., 1999). However, recently Falchi et al. (2006) discovered that both the flesh and skin of grapes are equally cardioprotective despite the fact that grape flesh does not possess any anthocyanin components. Whether Etn is found in both the skin and flesh is unknown but it may be the silent cardioprotective factor found in grapes.

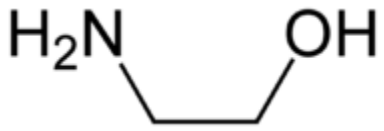


Figure 2: The chemical structure of ethanolamine making it both a primary amine and a primary alcohol.

Cultivar	Quality designation*	n	Ethanolamine (mg/L)
Musts			
Riesling	70°Oe	2	5 - 6
Müller-Thurgau	64 - 71°Oe	6	5 - 8
Grauburgunder	88°Oe	1	4
Weissburgunder	80°Oe	1	5
"Weissherbst"	76 - 82°Oe	2	3 - 6
Wines			
Riesling	QbA - Kab	5	12 - 16
Gutedel	QbA	3	12 - 17
Müller-Thurgau	QbA - Kab	3	6 - 11
Grauburgunder	QbA	2	7 - 8
Weissburgunder	QbA - Kab	3	5 - 8
Nobling	QbA	1	6
Silvaner	QbA	1	5
"Weissherbst"	QbA	1	6
"Rotgold"	QbA	1	4
"Rotwein"	QbA	1	4

* °Oe = Degrees Oechsle. QbA = Qualitätswein bestimmter Anbaugebiete (wine from certain area), Kab = Kabinett (type of qualified wine).

Table 1: The quantity of ethanolamine found in various wines and grape musts (Pfeiffer and Radler, 1992).

2.4.1.1 Cardioprotection by Ethanolamine

Previous research showed that pre-treatment with Etn protects the isolated rat heart from ischaemia-reperfusion injury when administered chronically and acutely (Kelly et al., 2010). This protection occurred via activation of signal transducer and activator of transcription 3 (STAT-3) as well as via activation of the survival activating factor enhancement (SAFE) pathway, a pathway that can be activated by pro-inflammatory cytokines (Lecour et al., 2002; Lecour, 2009). The Janus Kinase (JAK)/STAT-3 pathway is also known to be involved in the development of cardiac hypertrophy (Kunisada et al., 1998). Kume et al. (2006) discovered that Etn decreased serum cholesterol levels (particularly very low density lipoproteins and low density lipoproteins) in a rat model of hypercholesterolemia. Hypercholesterolemia is a risk factor for MI and subsequent HF. The mechanism by which Etn induced protection occurs is uncertain but Kume et al. (2006) state that Etn can lower serum cholesterol levels by suppression of apolipoprotein B messenger ribonucleic acid expression in the liver. Etn and its plasmalogens may also prevent free radicals oxidising

cholesterol and therefore could be cardioprotective via its anti-oxidant abilities (Maeba and Ueda, 2004; Vance, 1990). Etn is also known to affect the renin-angiotensin-aldosterone system functioning, a system that modulates BP (Pfeiffer et al., 1971).

2.4.2 Magnesium

Mg^{2+} and Ca^{2+} are two important divalent cations that regulate cellular activity. Mg^{2+} is known to be involved in over 300 enzymatic reactions in the human body including ATP metabolism. In the diet, Mg^{2+} is common in legumes, nuts, animal protein and green leafy vegetables (Whang et al., 1994). A deficiency of Mg^{2+} results in an intracellular loss of Na^{+}, K^{+} and Ca^{2+} which has been associated with a host of cardiovascular disorders including: diabetes mellitus, hypertension, arrhythmias, atherosclerosis and acute MI (Rardon and Fisch, 1994; Resnick, 1997; Fox et al., 2001). Mg^{2+} is used in cardiovascular medicine to manage arrhythmias and electrocardiogram (ECG) waveform abnormalities (Kleinfeld and Gross, 1956; Rickets et al., 1969; Rasmussen et al., 1986; Ceremuzyuski et al., 1989; Gwanyanya et al., 2004) and hypertension (Yusuf et al., 1993), inhibit thrombus formation (Shechter et al., 1995) and promote mitochondrial synthesis (Hearse et al., 1977; Rasmussen et al., 1988). Mg^{2+} is also used in the management of post-MI dysfunction by modulating Ca^{2+} (Meissner and Henderson, 1987; Altura and Altura, 1987; Rasmussen et al., 1988; Rasmussen et al., 1988; Woods, 1991; Jin et al., 2007) and serving as an anti-oxidant (Garcia et al., 1998). The importance of Mg^{2+} in the human body, and in particular for cardiac function, is highlighted by the study of Whang and Ryder (1990) who found that 42.2% of hospitalised patients were hypomagnesemic.

2.4.2.1 Magnesium and Cardiovascular Diseases

There is an inverse relationship between the amount of Mg^{2+} present in drinking water (water-hardness) and CVD which has been studied for over three decades (Peterson et al., 1970; Comstock, 1979; Rubenowitz et al., 1999). Atherosclerosis Risk in Communities (ARIC) was a longitudinal study that followed 15 000 patients over a five year period and investigated the dietary intake of Mg^{2+}, serum Mg^{2+} levels, race and correlated it to a predisposition to hypertension, diabetes and atherosclerosis. The results indicated that African Americans consumed lower dietary Mg^{2+} and therefore possessed lower serum

levels of Mg^{2+} leaving them at risk for developing cardiovascular complications (Ma et al., 2005). Another study involving 400 patients over a 10 year period recommended that high risk individuals for developing CAD should eat a Mg^{2+} rich diet to lessen the risk of sudden cardiac death, mortality, hypokalemia and overall cardiac dysfunction (Singh, 1990).

2.4.2.2 Hypomagnesemia

A deficiency in Mg^{2+} can be the result of alcoholism, disturbances of the gastrointestinal tract, endocrine disorders, renal disease and diuretic therapy and can lead to serious cardiac complications such as hypertension and pre-eclampsia, arrhythmias, dyslipidemia, acute MI and sudden cardiac death (Singh et al., 1976; Purvis and Movahed, 1992; Fox et al., 2001; Booth et al., 2003). Mg^{2+} deficiency is mostly asymptomatic and manifestations of hypomagnesemia occur through cardiac, neurological and metabolic complications (Fox et al., 2001).

2.4.2.3 The Effects of Magnesium on Hypertension

Aldosterone regulates BP and it is common that patients with hyperaldosteronism will suffer from hypomagnesemia (Paravicini et al., 2009). The reasons for the association is unclear however, Sontia et al. (2008) suggest that movement of Mg^{2+} through the membrane channels transient receptor potential melastatin 6 and 7 (TRPM6 and 7), which are known to regulate the levels of Mg^{2+} in the body, may be impaired. A reduction in the expression of TRPM7 channels can lead to vasoconstriction, a complication common to hypomagnesemic patients (Zhu et al., 2011). The vasoconstriction results in decreased amounts of oxygen and nutrients delivered to the cardiac myocytes which could lead to MI (Chakraborti et al., 2002). Mg^{2+} also regulates ion movement across channels (Ca^{2+}-activated K^+ channels) and cellular pumps (Na^{2+}-K^+-ATPase) altering vascular tone peripherally. Therefore hypomagnesemia may disrupt ion movement, augmenting Ca^{2+}-dependant vasoconstriction (Fox et al., 2001). Hypomagnesemia also increases arterial stiffness in hypertensive patients, thus exacerbating the condition (Resnick et al., 1997). Treatment with Mg^{2+} is a known therapeutic modality for pre-eclampsia. Administration of Mg^{2+} causes release of prostaglandin (a potent arterial vasodilator) which increases the coronary blood flow and

decreases the peripheral resistance, lessening the afterload on the myocardium and limiting cardiac dysfunction (Watson et al., 1986; Seelig, 1994).

2.4.2.4 Magnesium and Arrythmias

Hypomagnesemic patients display prolonged QT corrected for heart rate (QTc) intervals, low amplitude T-waves and ST-segment depression and are prone to ventricular arrhythmias which are often fatal (Cohen et al., 1984; Laban and Charbon et al., 1986; Seeling, 1989; Adamopoulos et al., 2009). Mg^{2+} affects the automaticity and conduction of the myocardium due to its modulating effects on Na^{2+} and K^{+} fluxes across cell membranes. As early as 1970, Seller et al. conducted an experiment on digitalis-induced cardiotoxicity in canines. It was found that digitalis blocked the Na^{+}-K^{+}-ATPase pump which resulted in severe arrhythmias. Treatment with Mg^{2+} abolished these arrhythmias. In patients with Torsades de pointes (polymorphic ventricular tachycardia), Mg^{2+} therapy is often the only solution to terminate the arrhythmias (Abraham et al., 1987; Tzivoni et al., 1988; Nishimoto et al., 2012).

2.4.2.5 Magnesium and Acute Myocardial Infarction

The importance of Mg^{2+} as a therapy in suspected MI cases is supported by studies demonstrating that Mg^{2+} can decrease infarct size and oxygen demand (Altura, 1988; Morton et al, 1984). There is controversy regarding the levels of Mg^{2+} pre-infarction (Singh et al., 1983; Speich et al., 1988; Pereira et al., 1988). However, Jeppesen et al. (1986) analysed Mg^{2+} levels post-infarction and discovered that there was a Mg^{2+} deficit. Therefore the association between Mg^{2+} and MI requires more research. During MI the myocardium undergoes acidosis, decreasing cellular Mg^{2+} levels and increasing Ca^{2+} overload (Woods, 1991). Excess Ca^{2+} in the cell results in necrosis and apoptosis. Mg^{2+} has long been deemed "nature's physiological Ca^{2+} blocker" (Altura and Altura, 1987) and blocks the voltage-sensitive Ca^{2+} channel, attenuating the influx of Ca^{2+} into the myocardial cell and lessens the damage incurred by ischaemia (Yamaoka and Seyama, 1996; Jin et al., 2007). Mortality resulting from MI is dependent upon the extent of myocardial necrosis and research conducted on canines in a coronary occlusion model of MI displayed that a therapeutic dose of Mg^{2+} can reduce infarct size (Harnarayan et al., 1970; Christensen et al., 1995; Barros et

al., 1995). It has also been identified that individuals who have died from sudden cardiac death (coronary thrombosis or myocardial degeneration) have low levels of serum Mg^{2+} (Chipperfield et al., 1973). There are various mechanisms proposed by which Mg^{2+} protects against MI including its antitachydysrhythmic properties, inhibition of platelet aggregation, inducing arterial vasodilation to reduce afterload on the heart, increasing the energy production in the myocardium by improving mitochondrial ATP synthesis, as an anti-oxidant and by decreasing the catecholamine-induced Mg^{2+}-Ca^{2+} shifts (Hearse et al., 1977; Rasmussen et al., 1986; Ceremuzyuski et al., 1989; Yusuf et al., 1993; Shechter et al., 1995; Garcia et al., 1998) and as an antagoniser to the effects of β-adrenergic stimulation (Barros and Pileggi, 1991).

Two major studies, The Second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2, 1992) and The Fourth International Study of Infarct Survival (ISIS-4, 1996) provide differing evidence as to the role of Mg^{2+} as a cardioprotective agent. The conclusion drawn suggests that the time of Mg^{2+} administration is a critical factor (Seeling et al., 1996). Therefore many authors conclude that Mg^{2+} therapy is only beneficial when administered early after MI and in conjunction with reperfusion therapy (Leor et al., 1995). The aim of this study is to investigate whether Mg^{2+} can protect the rat heart *in vivo* pre-MI.

2.5 Neurological Dysfunction

MI is often followed by an increased risk of anxiety (Dickens et al., 2006) and depression (Frasure-Smith et al., 1995; Echols and Conner, 2010; Larsen et al., 2010). Anxiety and depression may also have negative impacts in terms of morbidity and mortality after MI (Frasure-Smith et al., 1995; Carney et al., 2004; Larsen et al., 2010). However, some authors argue that depression and anxiety do not predict mortality outcome after MI but may only affect the patient's quality of life after MI (Lane et al., 2001). It is unclear if the depression and anxiety experienced is a physiological response to MI and HF; or if the emotional burden of being hospitalised and the fact that depressed individuals are less likely to adhere to treatment regimes, may be contributing to the increased levels of anxiety and depression accompanying heart disease (Ziegelstein et al., 1998). Recently studies on rats are becoming important in deciphering the neurobiochemical pathways of depression post-MI, and Wann

et al. (2007) suggests that an apoptotic pathway is activated after MI which may accelerate the development of depression. The development of anxiety-like behaviours in mice has also been linked to oxidative stress pathways (Hovatta et al., 2005; Bouayed et al., 2007).

2.5.1 Pathophysiology of Depression

Depression is a prevalent disorder of mood that causes impedance to an individual completing their day-to-day undertakings (Seligman and Reichenberg, 2011). Depression is manifested through behavioural deficits, central monoamine abnormalities as well as HPA axis activation. All these factors alter immune function, causing an impairment of zymosan-induced neutrophil phagocytosis, alterations in the natural killer cell activity, activated T-cells, mitogen-stimulated lymphocyte proliferation, increase in the blood lymphocyte, neutrophil and monocyte content, an increase in the serum positive acute-phase proteins as well as a decrease in the negative acute-phase proteins and an increase in the secretion of cytokines (Pasic et al., 2003; Szczepanska-Sadowska et al., 2010). Increased levels of inflammatory cytokines are positively correlated with depression and anxiety in humans and depressive-like behaviour in animals (Himmerich et al., 2008; Goshen et al., 2008; Dean et al., 2010; Sanders and Maze, 2010). After ischaemic injury there will be an augmentation of cytokines and oxidative stress, due to activation of NF-κB, circulating in the central nervous system and the limbic system (Matsui et al., 1999; Rousseau et al., 2012). These cytokines have access to brain tissue and cause apoptosis in the limbic system (Wann et al., 2006), decreased neurogenesis due to an interaction with brain-derived neurotrophic factor (Kaloustian et al., 2008), and an alteration in the metabolism of neurotransmitters (Dunn, 1992). The contributions of decreased neurogenesis and activation of the HPA axis (and subsequent alterations in brain metabolism) post-MI depression are still relatively unclear and require further investigation (Rousseau et al., 2012).

2.5.1.1 Rat Studies on Depression

During reperfusion in the rat, cytokines are released and it is thought that the cytokines bind to receptors in various brain areas such as the hippocampus, amygdala and the hypothalamus (Vitkovic et al., 2001; Francis et al., 2004). Cytokines play a major role in the pathophysiology of depression (Pasic et al., 2003). Other mechanisms which have been

shown to induce depression include: dysfunction of mitochondria and electrolyte disturbances (Baer et al., 1970; Barraclough, 1997; Kato and Kato, 2000).

2.5.2 Pathophysiology of Anxiety

When an individual feels threatened due to an inability to predict or control the outcome of situations, they experience a negative affective state termed anxiety (Barlow, 1988). There has been little research conducted on whether anxiety is independently a risk factor for coronary artery disease (CAD). Anxiety measures can predict incidence of MI, non-fatal MI, fatal CAD and sudden cardiac death (Kawachi et al., 1994; Kubzansky et al., 1997; Shen et al., 2008). However, some authors still argue that in patients with MI it is depression, not anxiety, which negatively influences the autonomic control of heart rate (Hippisley-Cox et al., 1998; Pitzallia et al., 2001). Patients with elevated anxiety but with no incidence of CVD displayed increased levels of fibrinogen, interleukin-6, tumour necrosis factor-α (TNF-α), homocysteine and C-reactive protein and female patients specifically also exhibited increased white blood cell count (Pitsavos et al., 2006). Therefore the authors concluded that anxiety may increase the risk of CVD through inflammation and coagulation pathways. The pathophysiology of anxiety and heart disease has also been linked to an increase in circulating catecholamines, platelet aggregation, myocardial demand for oxygen as well as vasospasm (Kawachi et al., 1996). Further evidence that anxiety may occur via a coagulation and therefore thrombus formation pathway, include studies on aspirin. Aspirin has been known to alleviate anxiety levels possibly due to the anti-platelet aggregation effects (Mittleman et al., 1995).

2.5.2.1 Rat Studies on Anxiety

The development of anxiety-like behaviour in mice has been linked to increases in oxidative stress in neuronal cells (Hovatta et al., 2005; Rammal et al., 2008). Recently it is noted that oxidative stress in the peripheral blood granulocytes can also contribute to the development of anxiety in a rat (Bouayed et al., 2007). There is also evidence to suggest that components of the renin-angiotensin-aldosterone system are implicated in anxiety disorders in rats (Saavedra et al., 2005).

2.5.3 Cracking the Chicken and Egg Dilemma

As mentioned previously, emotional stress and subsequent activation of the HPA system as well as the sympathoadrenal system can trigger the occurrence of a severe cardiomyopathy (Ueyama et al., 2008). The make-up of that emotional stress, being either predominantly depression or anxiety, is not clear. Research is needed to monitor both anxiety and depression after MI. It is known that cardiac arrest can affect cognitive functioning via transient brain ischaemia (Kiryk et al., 2011). Whether depression and anxiety are risk factors for MI and subsequent HF (Konstam et al., 2005), or if MI physiologically causes depression and anxiety require much research (Pederson et al., 2010). Konstam et al. (2005) portrayed that depression and anxiety may cause cardiac disturbances (Fig. 3 shown in blue), yet the authors did not illustrate that cardiac dysfunction in turn may impact the anxiety and depression levels of the patient as demonstrated by Grippo and Johnson et al. (2002) (Fig. 4 shown in red). Grippo and Johnson (2002) similarly did not accept that altered mood changes may directly cause cardiac events. Thus evidence suggests there is an unclear overlap between cardiac injury and brain dysfunction.

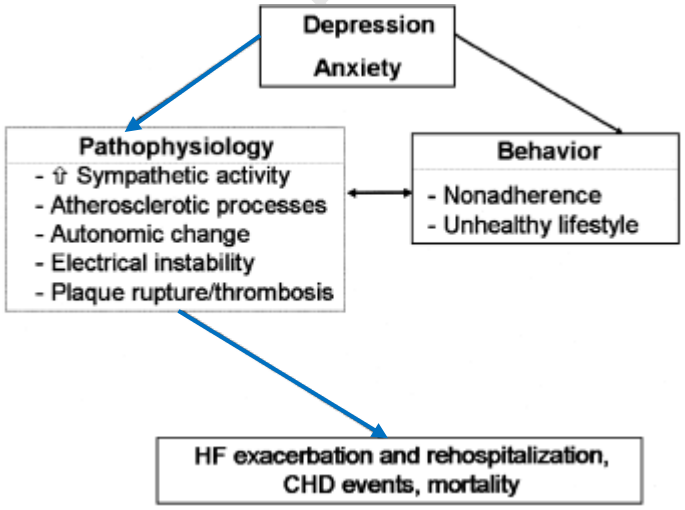


Figure 3: The pathophysiology of depression, anxiety and cardiovascular dysfunction as portrayed by Konstam et al. (2005).

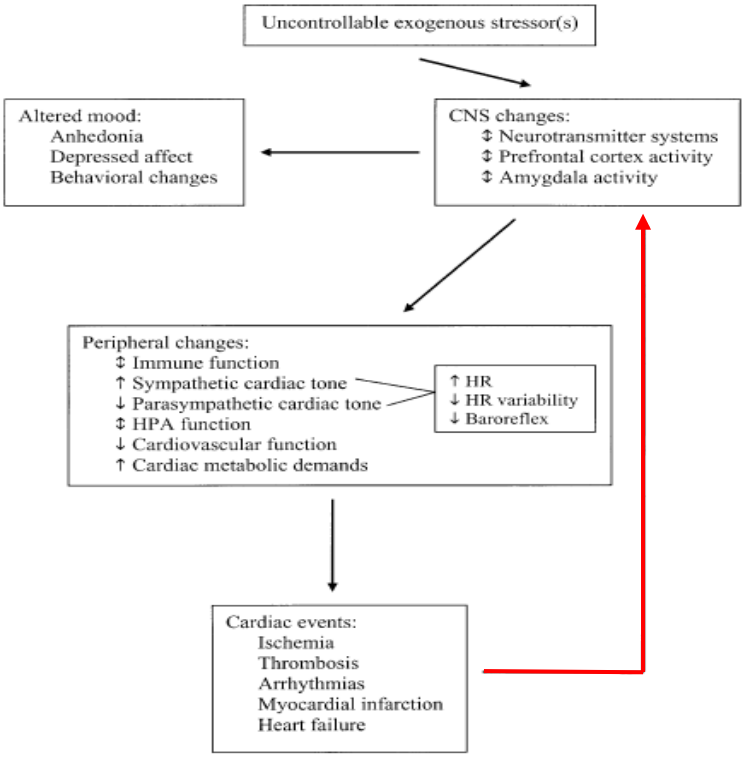


Figure 4: Grippo and Johnson's (2002) portrayal of the pathophysiology of behavioural alterations and the interaction with cardiac events.

2.5.4 Substances Used in the Treatment of Cardiovascular Disease

The aspirin study conducted by Mittleman et al. (1995) suggest that the effects of cardioprotective substances on the prevention and minimisation of damage after MI may not strictly be limited to direct interactions with cardiac-specific parameters, but may alter mood states which indirectly protect from cardiac dysfunction. Recently Anwar et al. (2011) tested Alprazolam (a potent anxiolytic) in a mouse model of HF (doxorubicin) and found that anxiety was reduced in mice when Alprazolam was administered post-infarction. Administering the anxiolytic pre-infarction resulted in less cardiac damage from the doxorubicin. The mechanism by which this cardioprotection occurred was via reductions in lipid peroxidation and lactate dehydrogenase (LDH) production. Berkowitz et al. (1987) used Alprazolam in a rat model of ISO-induced MI. Alprazolam protected against electrocardiographic dysfunction by maintaining well-structured T-waves and limiting the appearance of pathological Q-waves. Alprazolam also reduced infarct size and prevented the expected increase in heart weight to body weight (HW/BW) ratio that ISO induces. Thus a powerful anxiolytic can reduce cardiac dysfunction in animal models.

Prickaerts et al. (1996) treated rats post-MI with Captopril, a potent angiotensin-converting enzyme inhibitor that is used to manage hypertension in humans. The authors found that coronary artery ligation caused an increase in the anxiety state of the rat. When rats were treated with Captopril post-coronary artery ligation they exhibited a reduction in anxiety-like behaviour. The blood pressure (BP) modulating drug had to be administered at least three to five weeks after the infarction was induced, in order to lower anxiety levels of the rats. Interestingly, when control rats (without ligated arteries) were given Captopril, there was an opposite effect, in that the rats became more anxious. Therefore the use of cardiac modulating drugs in the management of affective disorders needs to be used with caution.

Depression post-MI can occur in a rat model of MI (coronary artery ligation). This can occur because of apoptosis in the limbic system (Wann et al., 2006). The anti-depressant, sertraline, prevents this apoptosis and can therefore limit depression post-MI (Flugy et al., 2008). This limitation of depression may stop progression to HF, if Konstam's et al. (2005) theory holds true in rat models. Therefore substances which affect behaviour and mood, administered before and after the onset of MI may provide cardioprotective effects. Cardioprotective substances should also improve mood disorders if Grippo and Johnson's (2002) theory holds true in animal models.

2.5.5 Neurological Protection by Ethanolamine

Matas et al. (2007) found that cultured neuronal cells were protected from apoptosis by the degradation of anandamide to Etn by fatty acid amide hydrolase (FAAH). Etn protected via activation of Caspase 3/7 and cleavage of poly (ADP-ribosome) polymerase-1. As depression and anxiety pathways are intimately linked with apoptosis in brain cells (Flugy et al., 2008), Etn may have an effect on behavioural changes. Interestingly, anandamide hydrolysis has proven to have anti-depressive effects in rats (Kathuria et al., 2002; Gobbi et al., 2005). Therefore conducting research on the downstream product of anandamide, Etn, may aid in determining whether anti-depressive therapy aimed at FAAH inhibition is useful. Ion channels have been implicated in anxiety and depression disorders (Kaster et al., 2005; Heurteaux et al., 2006). Etn is known to modulate voltage-activated K^+ channels and can interact with intracellular Ca^+ signalling which alters the sensory excitability of neuronal cells

(Khairy et al., 2010). The pathophysiology of affective illnesses include the dysfunction of mitochondria (Kato and Kato, 2000) and there is an unclear link between Etn and mitochondrial function (Modica-Napolitano and Renshaw, 2004). Nitsch et al. (1992) discovered that patients who suffered from Alzheimer's disease, a common form of dementia, had lower levels of Etn in the frontal, parietal and the primary auditory cortices. However there has been no research conducted on the effects of treating Alzheimer patients with Etn.

2.6 The Use of Animals in Cardiovascular Research

Animal models are widely used in cardiovascular research, particularly for the investigation of pharmacological interventions. Animal models are often reproducible and can be modified to mimic a variety of different cardiac conditions as well as the transition stages of CVD (Schwartz et al., 1997). Small animal models are becoming increasingly popular due to the cost-effectiveness of the husbandry associated with small animals (Schwartz et al., 1997). The most common animals used are: dogs, rabbits, mice and rats (Hasenfuss, 1998). Canines are often used in pacing studies to assess the progression of cardiac remodelling to HF (Travill et al., 1992; Eaton et al., 1995). While a canine model may be more accurate in representing left ventricular function than a rodent model, the husbandry and resources required may outweigh the accuracy (Hasenfuss, 1998). The rabbit as a model is less expensive than canines and the failing rabbit myocardium represents similar alterations as the human failing heart, making rabbit studies used more for end stage HF studies than for pre-MI treatment studies (Yamani and Massie, 1993). The most common species for cardiovascular research are the mouse and rat models.

2.6.1 The Rat as a Model for Cardiovascular Disease

Both the rat and mouse are small animals commonly used for cardiovascular research due to their cost-effectiveness, limited equipment required and the availability of the animals (Schwartz et al., 1998). The short gestation and life period allow for a large sample number and quick turn over of results and as rats and mice are used more frequently in recent science, it becomes possible to compare research more readily (Hasenfuss, 1998). Mice are also predominantly used for transgenic studies assessing gene overexpression, mutation

and knock out to isolate the genes and pathways surrounding HF progression, whereas the rat is more frequently used to test the effects of pharmacological substances (Iwase et al., 1997; Welikson et al., 1999; Arber et al., 1997). Mice are also smaller than adult rats, and therefore present more technical difficulties during experiments (Doggrell and Brown, 1998).

There are some limitations to using rat models for cardiac studies. Firstly, the rat exhibits a shorter cardiac action potential, compared to humans, with no noticeable plateau phase. On the ECG the S and T-waves seem to merge making identification of the J-point difficult (Bers, 1991; Farraj et al., 2011). Therefore, analysing the ECG characteristics of this model becomes slightly more difficult when transferring it to human pathology. Secondly, the rat has a resting heart rate that is five times faster than humans and exhibits a negative force-frequency relationship (Bers, 1991).

2.7 Rat Models of Myocardial Infarction

2.7.1 Coronary Artery Ligation

A common method to induce ischaemia is ligation of the left coronary artery in rats. This reduces blood flow to the myocardium and induces left ventricular impairment due to necrosis of cardiac tissue (Maroko et al., 1971). Complete ligation of the left coronary artery disrupts the electrophysiology, morphology, biochemistry, haemodynamics and the mechanical workings of the infarcted myocardium (Fishbein et al., 1978). The disruptions are similar to those in human MI, except that the MI evolves faster in the rat which may be due to the rat's smaller size and faster metabolism (Bing et al., 1956).

2.7.2 Cauterisation

Cauterisation is achieved by exposing the epicardium or arteries to a hot or freezing caustic agent to induce focal infarctions by reducing the coronary blood flow to the cauterised area. This damages the myocardium and cause coagulation of blood at the site of injury (Adler et al., 1975). Cauterisation is quick, inexpensive and is a reliable method for assessing the impact that interventions may have on infarct size (Adler et al., 1975; Staab et al., 1977).

However, unintentional damage to the myocardium when attempting to cauterise only the coronary artery is common (Moskowitz et al., 1979).

Surgical methods such as ligation and cauterisation produce localised infarcts but these techniques are invasive, requiring anaesthesia and thoracotomy. Thoracotomy alone without vessel ligation has been shown to have mortality rates as high as 18% (Johns and Olson 1954). The mortality associated with coronary artery ligation is 40-50% within 24 hours post-surgery (Pfeffer et al., 1979) and the infarct size varies (van den Bos et al., 2005).

2.7.3 Pharmacological Induction of Myocardial Infarction

In contrast to surgical methods, pharmacological tools can be used non-invasively to induce MI in the rat, but generally the infarcts induced are global and non-specific. Ethanol ingestion produces a model for cardiac dysfunction with varying degrees of infarct sizes, making it a better model for HF than MI (Capasso et al., 1992). Cocaine induces cardiac stress but its systemic side effects have limited the use of the model (Fineschi et al., 2001). Catecholamines are known to induce myocardial necrosis by creating an imbalance in the oxygen supply and demand ratio as well as the exhaustion of high energy phosphates and induction of intracellular Ca^{2+} overload (Rona et al., 1959; Fleckeinstein et al., 1974; Singal et al., 1982). The synthetic catecholamine ISO, induces dose-dependent gross and microscopic infarcts similar to those which occur in human MI (Rona et al., 1959; Baroldi, 1974). Although ISO administration does not mimic the sequence of events during an MI when the artery is blocked, as coronary artery ligation does, the damage incurred by ISO post-MI is similar to that which occurs in the human.

2.8 Rat Models of Cardiac Hypertrophy

Small animal models are also used to study the development and progression of cardiac hypertrophy. The rat is used most frequently.

2.8.1 Rat Strains

The spontaneously hypertensive rat develops hypertension-induced hypertrophy culminating in HF in the last 6 months of their 2 year life span (Mitchell et al., 1997). The

progression from cardiac hypertrophy to HF in this model follows a similar pathological development as in the human (Doggrell and Brown, 1998). Transgenic rats are more commonly being used as the pathogenesis of the disease is controlled and predictable. Murine Ren-2 has been isolated as the gene which modulates the renin-angiotensin system and thus, cardiovascular functioning (Lee et al., 1996). Altering angiotensin-II will increase BP and result in pathological cardiac hypertrophy (Doggrell and Brown, 1998).

2.8.2 Surgical Induction of Hypertrophy

Occlusion of the renal artery causes renovascular hypertension resulting in concentric left ventricular hypertrophy and ultimately cardiac failure. Because of the pressure overload, there is an increase in wall thickness without a corresponding increase in chamber size. Therefore the myocytes will increase in size laterally but not in quantity (Anversa et al., 1991). This produces a specific type of hypertrophy not always common to HF patients. Another surgical method involves banding of the aorta in which the outflow from the heart is restricted causing pressure loading to the myocardium resulting in hypertrophy (LeLievie et al., 1986). All surgical methods involve anaesthesia and thoracotomy or abdominal surgery which may have unpredicted adverse effects.

2.8.3 Pharmacological Induction of Cardiac Hypertrophy

Increased catecholamine circulation in the body, due to overstimulation of the sympathetic nervous system, can cause cardiovascular morbidity and HF (Cohn et al., 1984). A common model for left ventricular hypertrophy is epinephrine infusions. This correlates well with the human progression to HF due to activation of the sympathetic nervous system. Epinephrine can mediate α-adrenoreceptor vasoconstriction and is therefore often used as a model for chronic HF rather than for a model highlighting the development of hypertrophy. ISO, a non-selective β-adrenoreceptor agonist, causes the development of a left ventricular chamber enlargement that is out of proportion to an increase in mass, similar to that which occurs in human pathological hypertrophy (Teerlink et al., 1994; Doggrell and Brown, 1998).

2.9 Isoprenaline-induced Cardiac Dysfunction

Isoprenaline hydrochloride [1-(3,4-dehydroxyphenyl)-2-isopropylaminoethanol hydro-chloride], an analogue of epinephrine, is a synthetic catecholamine as well as a β-adrenergic receptor agonist (Rona et al., 1959; Nirmala and Puvanakrishnan, 1994). ISO is synthesised by adding isopropylamine to ω-chloro-3,4-dihydroxyacetophenone and removing a carbonyl group (Fig. 5). There is a close correlation between the dose of ISO injected and the extent of damage to the myocardium, therefore ISO can induce both gross and microscopic infarcts similar to that which occurs in human MI (Rona et al., 1959; Baroldi, 1974; Sharma et al., 2001). When catecholamines are administered at high doses, there is an influx of Ca^{2+} into the cell and the energy stores of the cardiomyocytes are depleted resulting in irreversible damage through biochemical and structural alterations (Jennings et al., 1978; Rajadurai and Prince, 2007; Upaganlawar et al., 2011). ISO at supraphysiological doses induces apoptosis and necrosis of the myocardium as well as interstitial fibrosis, resulting in left ventricular dysfunction and subsequent hypertrophy of the left ventricle (Grimm et al., 1998; Shizukuda et al., 1998). ISO also causes β-adrenergic desensitization which can exacerbate cardiac dysfunction (Hertel and Perkins, 1984). Due to the infarction caused by ISO, there is a mortality rate associated with the model ranging from 25% to 50% (Judd et al., 1969; Wexler 1979; Crandall et al., 1981; Singal et al., 1982; Mladenka et al., 2009).

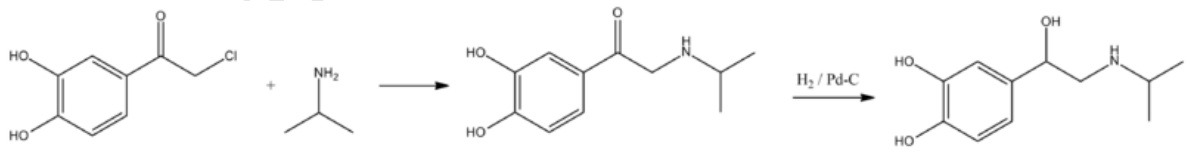


Figure 5: Isoprenaline is a synthetic catecholamine with a similar chemical structure to epinephrine (Schening and Thomae, 1943, US Patent 2,308,232).

2.9.1. Mechanisms of Isoprenaline-Induced Cardiac Dysfunction

The mechanisms by which ISO causes MI are currently still debated. Proposed mechanisms include: myocardial hyperactivity, overload of Ca^{2+} and the generation of free radicals (Rona et al., 1959; Singal et al., 1982; Mohanty et al., 2004).

2.9.1.1 Myocardial Hyperactivity

Administration of ISO causes peripheral vasodilation, lowering BP. Due to ISO's positive inotropic and chronotropic effects, administration causes coronary hypotension and induces reflex tachycardia, increasing the demand for oxygen by the myocardium. The demand is not met because of the lowered BP reducing the amount of blood available to the working myocardium, resulting in ischaemic necrosis similar to that which occurs in human MI (Rona et al., 1959; Nirmala and Puvanakrishnan, 1994; Rajadurai and Prince, 2007). At a cellular level, the necrosis can be viewed as well as the separation of cardiomyocytes and an infiltration of inflammatory cells (Kumar et al., 2009; Patel et al., 2010). The ischaemia causes an overload of Ca^{2+} in the myocardial cells (Nirmala and Puvanakrishnan, 1994).

2.9.1.2 Calcium Overload

The overload of Ca^{2+} causes a depletion of high energy phosphate stores by activation of the plasma membrane Ca^{2+}-dependant ATPase pump. This inactivates the Na^+/K^+ ATPase pump, inhibiting vital Na^+ and K^+ transport (Rajadurai and Prince, 2007). High energy phosphate depletion results in ultra structural changes to the myocardial cells, altering functional capabilities (Jennings et al., 1978). Increasing intracellular Ca^{2+} causes cell swelling and leakage of myocardial enzymes and contractile proteins culminating in cell death. Interestingly, when Ca^{2+} channel blockers are administered, the injury caused by ISO is significantly lessened (Kawai et al., 1998).

2.9.1.3 Generation of Free Radicals

Auto-oxidisation of ISO generates highly cytotoxic free radicals such as quinones (Nirmala and Puvanakrishnan, 1994; Mukherjee et al., 2010). Reaction of quinones with oxygen, produces free radicals such as superoxide anion radical ($O_2\bullet$-) and hydrogen peroxide (H_2O_2). $O_2\bullet$- causes the liberation of iron from tissue and the production of hydroxyl radicals

(•OH), both of which initiate lipid peroxidation in the cells (Halliwell and Gutteridge, 1985; Biemond et al., 1986; Rubanyi and Vanhoutte, 1986). The free radicals are able to interact with lipids, proteins and nucleic acids resulting in lipid peroxidation of the plasma membranes, enzymatic disturbances and damage to DNA (Rajadurai and Prince, 2007). If these alterations are not rectified, cell death will occur (Singh and Chopra, 2004). Peroxidation of the lipid membrane can cause intracellular Ca^{2+} overload and cardiotoxicity (Bhandari et al., 2008). Lipid peroxidation decreases the activity of Na^{+}/K^{+} ATPase as well as Mg^{+} ATPase activity, causing ion imbalances and subsequent cardiac damage (Upaganlawar et al., 2009). When the DNA is damaged, this may affect the repair and transcription processes of the cell which exacerbates the disease process (Rajadurai and Prince, 2007). Nandave et al. (2009) found that ISO decreases activity of anti-oxidant enzymes such as: superoxide dismutase, catalase and glutathione peroxidise. With less anti-oxidant enzymes available, the myocardium is more vulnerable to lipid peroxidation. There is still uncertainty in the literature regarding the immediate effects of ISO on the extent of lipid peroxidation as both the quantity and frequency of ISO administration affects lipid peroxidation. Rathore et al. (1998) found that when injecting multiple doses of ISO, three hours after the second dose there is an increase in lipid peroxidation. But at 12 hours, the peroxidation levels return to control levels. When a single dose of ISO is injected there often can be a decrease in lipid peroxidation levels seen up to two days post-injection. As ISO administration initially causes oxidative stress, there will be hypertrophy of the myocardium to assist with adaptation to this stress. As hypertrophy is associated with an increase in the quantity of anti-oxidant enzymes, a decrease in lipid peroxidation levels may be common after a single dose of ISO (Singal et al., 1982; Singh et al., 1995; Rathore et al., 1998). Therefore the extent of lipid peroxidation may be time, dose and frequency-dependant.

2.9.2 Cardiovascular Effects of Isoprenaline Administration

ISO exerts both inotropic and chronotropic effects on the heart and causes necrosis of the myocardium, myocardial edema, ground substance accumulation or hypertrophy (Judd et al., 1969; Benjamin et al., 1989; Teerlink et al., 1994). ISO damages myocardial membrane integrity which causes the infiltration of inflammatory cells and disrupts electrolyte homeostasis (Kahn et al., 1969; Kumar et al., 2009; Patel et al., 2010). There is mitochondrial

swelling and calcification when ISO is administered at low doses frequently (Ferrans et al., 1964; Bloom and Cancilla, 1969). ISO causes an elevation of lipid peroxidation markers and retards anti-oxidant functioning (Wexler and Greenberg, 1978; Sharma et al., 2001; Karthikeyan et al., 2007; Mukherjee et al., 2010; Patel et al., 2010).

2.9.2.1 Electrophysiological Disruption in Isoprenaline-Induced Myocardial Infarction

Figure 6 shows the standard human ECG waveform. The P-wave represents atrial depolarisation; the QRS complex represents ventricular depolarisation with the T-wave being indicative of ventricular repolarisation. The U-wave is often present in healthy patients however; generally the U-wave represents electrolyte disturbances. The PR interval is indicative of the time between the onset of atrial depolarisation and the onset of ventricular depolarisation. The QT interval represents the time between the onset of ventricular depolarisation and the end of ventricular repolarisation. The ST segment represents completed ventricular depolarisation. The rat ECG waveform closely resembles the human ECG waveform except that rat waveform does not display a J-point (marking the end of the QRS complex and the beginning of the T-wave) and in non-diseased rats, the Q-wave is absent. All other parameters are similar to the human. ISO administration affects the electrophysiological functioning of the heart particularly by the induction of an anterior, evolving MI with accompanying myocardial edema, viewed by large Q-waves and decreased S-waves (DePace et al., 1983; Ramesh et al., 1998; Thygesen et al., 2007). The onset of MI is early after ISO administration and can be viewed by a decreased S-wave (Ekmekci et al., 1961). ISO also affects repolarisation of the ventricles as shown through disrupted T-wave and T_{peak}-T_{end} parameters (Antzelevitch et al., 1999; Pope et al., 2000).

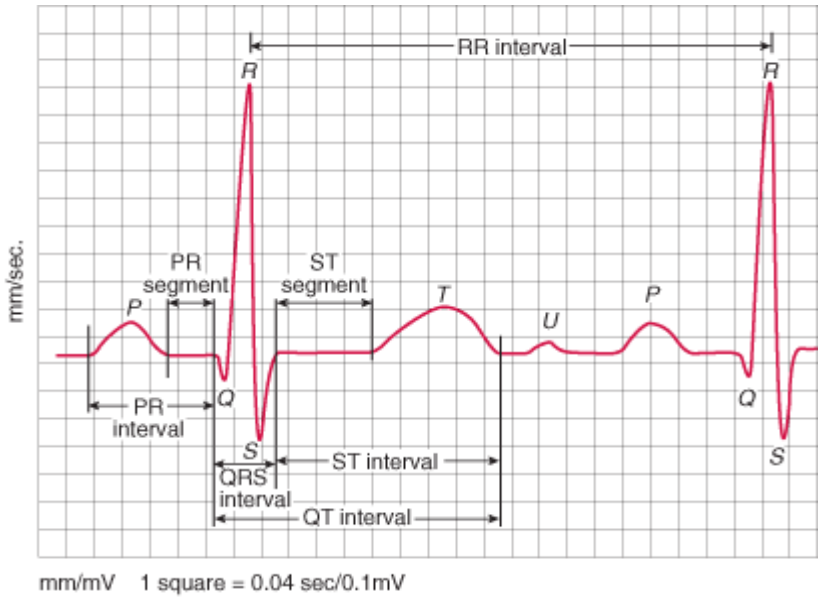


Figure 6: Standard human ECG waveform (The Merck Manual, 2009).

2.9.2.2 Blood Pressure Dysfunction in Isoprenaline-Induced Myocardial Infarction

In the human, the average blood pressure is between 75-125mmHg. The blood pressure waveform (Fig. 7) includes the systolic pressure (maximum arterial pressure), diastolic pressure (minimum arterial pressure) and the dicrotic notch (dicrotic wave) which represents the recoil of the blood against the closed aortic valve and is diminished in hypertensive patients (Feinberg et al., 1958). The arterial blood pressure waveform of the rat is similar to the human except that the systolic blood pressure of a non-diseased rat ranges from 84-134mmHg, with the diastolic blood pressure reaching 60mmHg (Wolfensohn and Lloyd, 2003). The blood pressure waveform of the left ventricle in both the human and rat also comprises of a maximum pressure and a minimum pressure however, the minimum pressure will be near to a zero value as majority of the blood is expelled from the ventricle during systole. Chappel et al. (1959) state that ISO causes peripheral vasodilation that manifests as systemic hypotension. Mohanty et al. (2004) confirmed that ISO causes a decrease in the arterial systolic blood pressure. ISO administration in a model of MI causes a rise in left ventricular end diastolic pressure (LVEDP), decreases contractility, and decreases the maximal and minimal rate of pressure change in the left ventricle (Mohanty et al., 2004; Jia et al., 2006; Zhou et al., 2008; Ojha et al., 2010). The alterations in diastolic function,

preload and afterload occur immediately after ISO administration as shown by Filipsky et al. (2012) who monitored haemodynamic changes 30s post-ISO infusion.

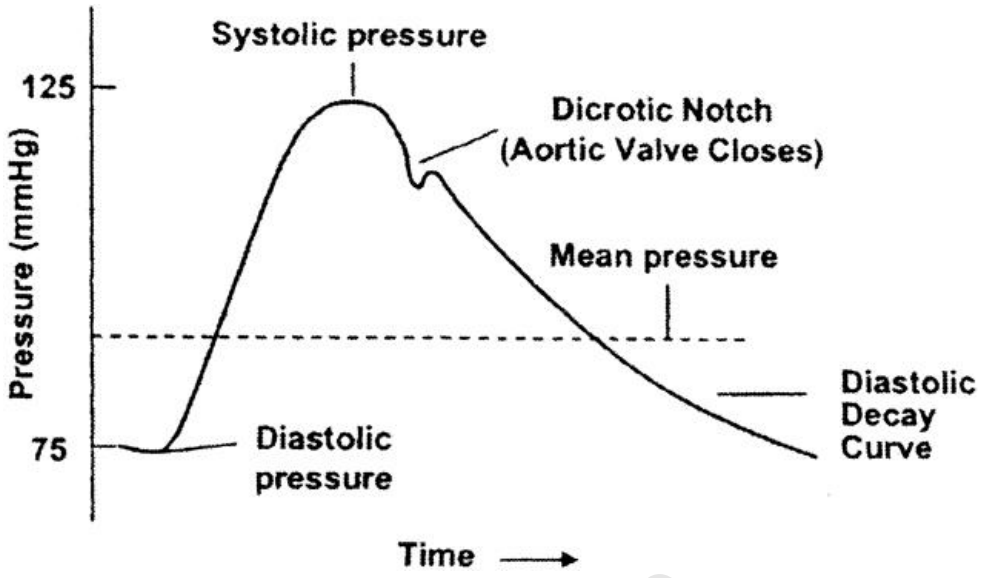


Figure 7: Human arterial blood pressure waveform (Syeda et al., 2003).

2.9.2.3 Systemic effects of Isoprenaline-Induced Myocardial Infarction

Within minutes of ISO administration, most rats became inactive, experienced respiratory distress and had some facial stains of mucosal bleeding before recovering (Wexler and Kittinger, 1963; Wexler et al., 1967). Within 24 hours, ISO alters protein metabolism and causes a loss of appetite (Wexler, 1971; Lora-Vilchis et al., 1988; Yamashita et al., 1994). Systemically, ISO alters organ functioning (Rona et al., 1959; Kahn et al., 1969). In the liver, ISO may cause necrosis and lipid accumulation (Pariza et al., 1977; Wexler, 1979; Grimm et al., 1998). ISO causes congestion in the lungs (Kahn et al., 1969), but the development of pulmonary edema that accompanies heart complications is not confirmed in the ISO model as it is in coronary artery ligation (Pasternak et al., 1992; Young et al., 1998; See et al., 2004).

2.9.2.4 Electrophysiological Effects of Isoprenaline-Induced Cardiac Hypertrophy

Chronic ISO administration causes cell membrane dysfunction which in turn can alter ECG characteristics (Tomaselli and Marban, 1999; Holland and Brooks, 1977; Kumar et al., 2009). ISO causes ventricular conduction abnormalities and can predispose the rat to developing arrhythmias as shown by prolonged QTc and a widened QRS and P-wave (Tang et al., 2011). Hypertrophy-induced ECG alterations include prolongation of the QRS and QT intervals as well as disruptions to T-wave morphology (Yan and Antzelevitch, 1998; Gima and Rudy, 2002; Kohutova et al., 2006).

2.9.2.5 Blood Pressure Dysfunction in Isoprenaline-Induced Cardiac Hypertrophy

In 1987, Tang et al. stated that ISO administered chronically affected the maximum but not the minimum rate of pressure change in the left ventricle. In 2011, Tang et al. showed that ISO-induced hypertrophy causes left ventricular pressure alterations, in both the maximum and minimum rates of pressure change. Arthur and Belcastro (1997) also stated that ISO increased the maximum pressure in the left ventricle.

2.9.2.6 Systemic Effects of Isoprenaline-Induced Cardiac Hypertrophy

Chronic administration of ISO induces weight gain due to increased food consumption, unlike acute ISO administration (Geleon et al., 1988; Perez-Llamas and Zamora, 1991; Kudej et al., 1997). The chronic administration causes an increase in the HW/BW ratio which can be indicative of cardiac hypertrophy (Benjamin et al., 1989; Ma et al., 2005; McMullen and Jennings, 2007). ISO can also affect the weight of the kidneys (Teerlink et al., 1994). The effect of ISO on the weight of other organs is poorly researched. In the plasma, ISO increases lipid peroxidation (Geng et al., 2004; Jaiswal et al., 2010). Grimm et al. (1998) showed that ISO affects the renin-angiotensin-aldosterone system. This system is known to be involved in the progression of cardiac remodelling to HF.

2.9.2.7 Neurological Effects Associated with Isoprenaline Administration

Minimal research has been conducted regarding the effects of ISO-induced cardiac dysfunction on the depressive and anxiety states of the rat. In other models of cardiac dysfunction, such as coronary artery ligation, depression and anxiety occur post-cardiac insult (Schoemaker et al., 1991; Prickaerts et al., 1996; Wann et al., 2007). The pathophysiology of depression and anxiety post-MI is largely unknown, however links have been made to apoptosis in the limbic system (Wann et al., 2007), central and peripheral oxidative stress (Hovatta et al., 2005; Bouayed et al., 2007; Rammal et al., 2008), angiotensin-converting enzyme inhibition (Prickaerts et al., 1996) and the release of pro-inflammatory cytokines (Burger et al., 2001; Pasic et al., 2003; Rousseau et al., 2012). The effect of ISO on the content of neurotransmitters, which may regulate depression and/or anxiety, is largely unknown. A hypothesis exists which links brain noradrenergic systems to affective disorders (Bunney and Davis, 1965; Schildkraut, 1965), yet Pohl et al. (1987) reported that administration of ISO does not affect this system. However, ISO has been implicated in affecting long-term potentiation in young rats, but the effect was not observed in older rats (Parfitt et al., 1991). The possibility of ISO affecting neurotransmitter content and subsequent behaviour should not be disregarded.

2.10 Aims and Objectives

The general aims of the study were to use ISO-induced cardiac disease models to investigate cardiovascular and neurological manifestations of the diseases as well to evaluate the effects of potentially cardiac- and neuro-protective pharmacological agents.

The specific objectives were as follows:

(a) To develop and characterize an optimal model of ISO-induced acute MI in rats.
(b) To investigate the effects of Etn on ISO-induced acute MI.
(c) To investigate the effects of Mg^{2+} on ISO-induced acute MI.
(d) To study the effects of Etn on cardiovascular and neurological manifestations of a well-established, ISO-induced cardiac hypertrophy model.

MATERIALS AND METHODS

3.1 Animals

Adult male Wistar rats (250-300g) were obtained from the University of Cape Town Animal Unit. Only males were used in the study as females undergo a hormonal cycle and can respond differently to MI compared to males (Vaccarino et al., 2001). The control of hormonal aspects is particularly important in heart hypertrophy, considering the fact that some neurohumoral processes (eg. involving angiotensin and aldosterone) are implicated in triggering the process (Wright et al., 2008). We acknowledge that males also have fluctuating hormones, but less so than caused by the oestrus cycle. All rats were allowed to acclimatise for two days under standard laboratory conditions (12 hour light/dark cycle 06:00-18:00, 300 lux, 22 ±1°C). Both temperature and light intensity can affect the rat's sensitivity to drugs and other physiological parameters such as lipid peroxidation and circadian oscillations, therefore these conditions were monitored constantly (Crabbe et al., 1994; Subash et al., 2007). Rats were fed standard rat chow (Afresh Vention 1, RSA) and had *ad libitum* access to food and water. Anaesthesia and *in vivo* measurements were conducted in a separate laboratory to where the animals were housed. Experiments were approved by The Faculty of Health Sciences Animal Ethics Committee at the University of Cape Town and performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No.85 (23), revised 1996).

3.1.1 Animal Husbandry

Scheving et al. (1968) noted that the circadian phase of the rat determined the rat's sensitivity to drugs. There is conflicting literature which determines the optimal light intensity that rats should be exposed to in their standard living conditions. The literature ranges from 130 to 500 lux (Semple-Rowland and Dawson, 1987; Wasowicz et al., 2002; Subash et al., 2007). But the standard most commonly used for albino rats is 300 lux (Becker and Grecksch, 1995; Huber et al., 1998). Incorrect lux can alter the rats' hormonal cycle, circadian rhythm, sensitivity to drugs and may cause retinal damage and excess stress (Mora et al., 1996; Wasowicz et al., 2002; Subash et al., 2007). The florescent lights on the ceiling in the rat housing facility were placed on a dimmer switch and an additional illuminated

table was placed in the centre of the room to ensure the lux was equal on top and bottom cages. The illuminated table was also placed on a dimmer switch and calibrated to the 12 hour light/dark cycle. This allowed all the cages to receive 300 lux. It was also noted that when there were more or less cages placed in the rat housing facility, the light intensity at each cage changed. For this reason, strips of LED lights were fitted above each rack and placed on the dimmer switch and timer system. This allowed for the light intensity to remain at exactly 300 lux regardless as to the amount of rats housed at any one time. The temperature was also adjusted to account for the increased emittance of light.

3.2 Disease Model Injection Protocols

ISO was dissolved in saline at 20 mg/ml and injected (67 mg/kg, s.c.) once for the induction of MI. This dose of ISO was determined in a preliminary study (see Results Section 4.1). To induce cardiac hypertrophy, ISO was dissolved in saline at 2.5 mg/ml and injected (5 mg/kg, i.p.). The model to induce hypertrophy proposed by Meszaros (1992) involves seven consecutive intraperitoneal injections of ISO at 5 mg/kg each day. This method is widely used and the development and progression of left ventricular hypertrophy has been confirmed (Inamoto et al., 2000; Ennis et al., 2003; Hanada et al., 2008). ISO can be oxidized shortly after preparation; therefore, the ISO solution was prepared freshly before each injection.

3.3 Pharmacological Intervention Injections

Etn was diluted in saline at 100 mg/ml and injected (10 mg/kg, i.p.) two hours prior to ISO treatment. The optimal dose was chosen based on a preliminary experiment which assessed infarct size and ECG characteristics (see Results Section 4.2.1). Mg^{2+} was given as magnesium sulphate dissolved in saline at 100 mg/ml and injected at 270 mg/kg, i.p. as performed in other studies (Euser et al., 2007). The Mg^{2+} was also administered two hours before ISO treatment. This Mg^{2+} dose is equivalent to serum levels achieved in human studies and in the treatment of pregnant women experiencing eclamptic seizures (Pritchard, 1955; Hallak et al., 1994; Leveno and Cunningham, 1999). This dose is not known to cause major adverse effects.

3.4 Experimental Design

A total of 172 rats were used in four separate studies shown below in Fig. 8. For characterisation of the ISO model, 44 rats were divided into two groups: control (n=18) and diseased (n=26). The rats received either ISO (67 mg/kg) or vehicle (equivalent volume of saline). For the study of the effects of Etn on MI, 53 rats were divided into four groups: saline (n=12), ISO (n=20), ISO + Etn (n=14) and Etn (n=7). The rats received either Etn (10 mg/kg) or vehicle (equivalent volume of saline) two hours before receiving ISO (67 mg/kg) or vehicle (equivalent volume of saline). For the study on the effects of Mg^{2+} on MI, 35 rats were divided into four groups: saline (n=8), ISO (n=9), ISO + mg (n=10) and mg (n=8). All rats received either Mg^{2+} (270 mg/kg) or vehicle (equivalent volume of saline) two hours before receiving ISO (67 mg/kg) or vehicle (equivalent volume of saline). In both these studies rats were weighed before treatment and then 24 hours later, after which the rats were anaesthetised to obtain *in vivo* electrophysiological, haemodynamic and gross structural measurements. For the study of the effects of Etn on cardiac hypertrophy, 40 rats were divided into four groups: saline (n=11), ISO (n=11), ISO + Etn (n=10) and Etn (n=8). All rats underwent forced swim test (FST) habituation on Day one (Section 3.5.3). On Day two, the rats were weighed and behaviour was assessed (Section 3.5) and two hours later, rats received ISO (5 mg/kg) or vehicle (equivalent volume of saline).On Day three, rats were injected with either Etn (10 mg/kg, i.p.) or vehicle (equivalent volume of saline) and then two hours later rats received the second ISO (5 mg/kg, i.p.) injection. ISO injections were repeated at the same time every day for a further five days. On Day nine, rats were weighed and the depression and anxiety states of the rats were assessed again and four hours later, rats were anaesthetised and *in vivo* electrophysiological, haemodynamic and gross structural measurements were taken.

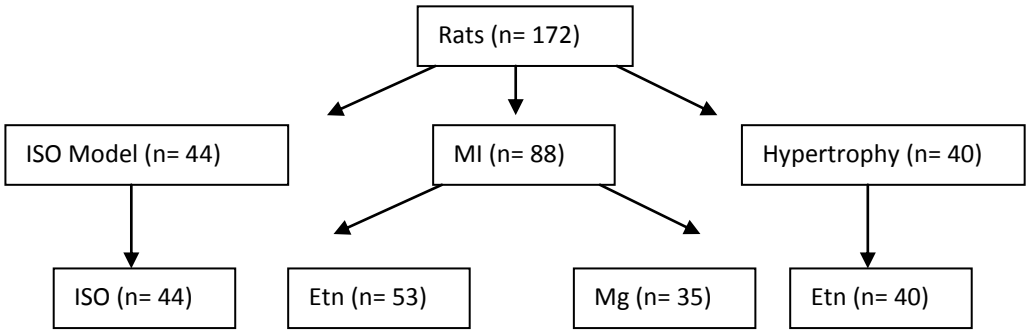


Figure 8: The experimental design to assess the impact of magnesium and ethanolamine on cardiac and neurological functioning in models of myocardial infarction and cardiac hypertrophy.

3.5 Behaviour Testing

Behavioural tests were conducted to monitor anxiety and depressive-like behaviour in the rat. Rats were allowed to habituate for one hour in the behaviour room prior to testing. The behaviour rooms were well-ventilated and maintained at 21-23°C with a light intensity of 150 lux. All behavioural apparatus (except for the FST) were cleaned with 70% ethanol after each trial. Before further testing resumed, the apparatus was dried to allow alcohol vapour to dissipate. All behavioural experiments were recorded with a video camera and analysed using Noldus Ethovision XT (Version 5, NED) (See Appendix 9.6).

3.5.1 The Elevated Plus Maze Test

The Elevated Plus Maze (EPM) is widely used as a test for anxiety in rats. It comprises two open arms (45 cm x 10 cm) and two closed arms (45 cm x 10 cm x 40 cm) that extend from a central platform (10 cm x 10 cm) elevated to a height 50cm above the floor. The rats were placed in the centre of the maze facing the open arm, and allowed to explore for five mins. Time spent in the centre zone, each arm and the frequency of entry into each arm were analysed. Reduced anxiety levels are displayed by a significantly more amount of time spent in the open arm as the closed arm provides shelter for the rat (Pellow et al., 1985).

3.5.2 The Open Field Test

Rats were tested in the open field for anxiety-like behaviour and locomotor activity. The Open Field (OF) test was performed in a wooden box measuring 100 cm x 100 cm x 50 cm. The box was divided into an inner and outer zone. This was demarcated by white tape on the floor 10 cm from the outer wall of the box. Rats were placed in the corner of the box and allowed to freely explore for five mins. Time spent and distance covered in the inner and outer zones of the box as well as the frequency of transition from the outer to the inner zones was analysed. Reduced anxiety is displayed by more time spent in the inner zone as well as increased locomotion due to the exploratory behaviour shown by the rat (Walsh and Cummins, 1976).

3.5.3 The Forced Swim Test

The FST is a well established model of depression in the laboratory rat. The original method is derived from Porsolt et al. (1978). During the FST rats were placed in a glass cylindrical tank with a height of 40 cm and a diameter of 19 cm, which was filled with water (25°C) to a depth of 20 cm. On the first day, rats were placed in the cylinder and left to swim for 15 mins (pre-test). The rats were placed in the cylinder individually and the water was replaced between each rat. After the 15 mins of swimming, the rats were removed from the cylinder and briefly dried with a towel before being placed back into their home cage. After 24 hours the above procedure was repeated, however the rats were only left to swim for five mins (test). These five mins of swimming behaviour provide an indication of depression. A non-depressed rat should display escape-like behaviour such as climbing and swimming and a depressed rat will float more (immobility) (Porsolt et al., 1978). The FST was evaluated manually using set criteria (See Appendix 9.7) as often Noldus software did not detect the rat accurately. The results were checked by an independent observer.

3.6 Care of Anesthetised Animal

Sodium pentobarbitone, belonging to the family of barbiturates, is a central nervous system depressant. The effects therefore can range from total anaesthesia to mild sedation. The pharmacokinetics of pentobarbitone include: a reduction in the blood-brain glucose transfer and hepatic metabolism and distribution (Ossenberg et al., 1975; Gjedde and Rasmussen,

1980; Knodell et al., 1980). In rabbits, cats and dogs, pentobarbitone administration decreases the rate and depth of respiration, induces hypotension and may cause alternate ventricular rhythm (Gruber et al., 1937). Pentobarbitone also inhibits parasympathetic reflex vasodilation (Ito et al., 1998). Sodium pentobarbitone (10% concentrate in saline) was injected (60 mg/kg, i.p.).

3.6.1 Ventilation

Once an appropriate depth of anaesthesia was confirmed (approximately five mins), the rat was intubated and mechanically ventilated with room air at a rate of 70 strokes/min and 2.5 ml room air/stroke on a rodent ventilator (Model 681, Harvard Apparatus, USA). A top-up dose of anaesthetic (12 mg/kg, i.p.) was administered if required. Depth of anaesthesia was determined by assessment of the pedal withdrawal reflex. If the rat did not respond adequately to the first dose, a top-up dose of 12 mg/kg (i.p.) would be administered before intubation.

3.6.2 Temperature Regulation

During anaesthesia rats were placed on a heating pad (37°C) and body temperature was monitored using a rectal probe (Physitemp, USA) connected to a data acquisition system (Powerlab 4/30, ADInstruments, Aus) via a T-type pod (ML312, ADInstruments, Aus). If the body temperature increased above or below the normal range for the rat (36°C to 37°C), the heating pad was temporarily switched on or off until the body temperature normalised.

3.7 *In Vivo* Electrophysiological and Haemodynamic Measurements

All electrophysiological and haemodynamic data recorded online by the PowerLab system were digitally acquired and analysed using the LabChart Pro 7 software (ADInstruments, Aus). The heart rate and ECG waveform were monitored from a lead II of a 3 lead surface ECG connected to the PowerLab system via an Animal Bio Amplifier (ML136, ADInstruments, Aus). ECG data were analysed using LabChart 7 Pro ECG Analysis Module software, preset to the rat ECG waveform (See Appendix 9.4). The Bazett formula was chosen to calculate QTc which states that $QTc=QT/RR^{0.5}$. The neck was superficially dissected to expose the right carotid artery. In some rats (n=75) the right carotid artery was cannulated with a

heparinised, custom-made cannula attached to a pressure transducer (MLT0670, Lasec, RSA) to measure arterial BP. Once inserted, the cannula was flushed with warm saline (0.1 ml) to remove any clotted blood from the cannula tip. In the remainder of the rats (n=35) the right carotid artery was cannulated with a Mikro-tip pressure manometer (SPC320, Millar, USA; Kindly provided by Prof Edward Johns) to obtain left ventricular pressure readings. The Mikro-tip pressure manometer was zeroed in water at ±37°C. Initially, the catheter had been zeroed at room temperature and in air and when it was inserted into the animal; the manometer would drift from zero. This was rectified by zeroing in water and at body temperature approximately 37°C. In addition, to avoid the intermittent drifting from zero, the manometer was cleaned with a physiological detergent (Terg-A-Zyme) after each use. The detergent removes any microscopic blood and tissue particles left on the catheter tip from the previous animal. The manometer was calibrated before being inserted into each rat. The calibration took place using a standard mercury sphygmomanometer connected to the catheter via a 3-way tap. The rats were injected with heparin (100 IU) after anaesthesia to ensure no blood clots would form around the manometer tip. BP readings were recorded by the PowerLab system via a Bridge Amplifier (ML221, ADInstruments, Aus). The arterial and left ventricular BP data were analysed using LabChart 7 Pro BP Module software, from where parameters such as the left ventricular relaxation time constant, Tau, were derived (See Appendix 9.5). ECG and BP recordings took place 20 mins post-cannulation of the right carotid artery. The set-up of a measurement procedure is shown in Figure 9.

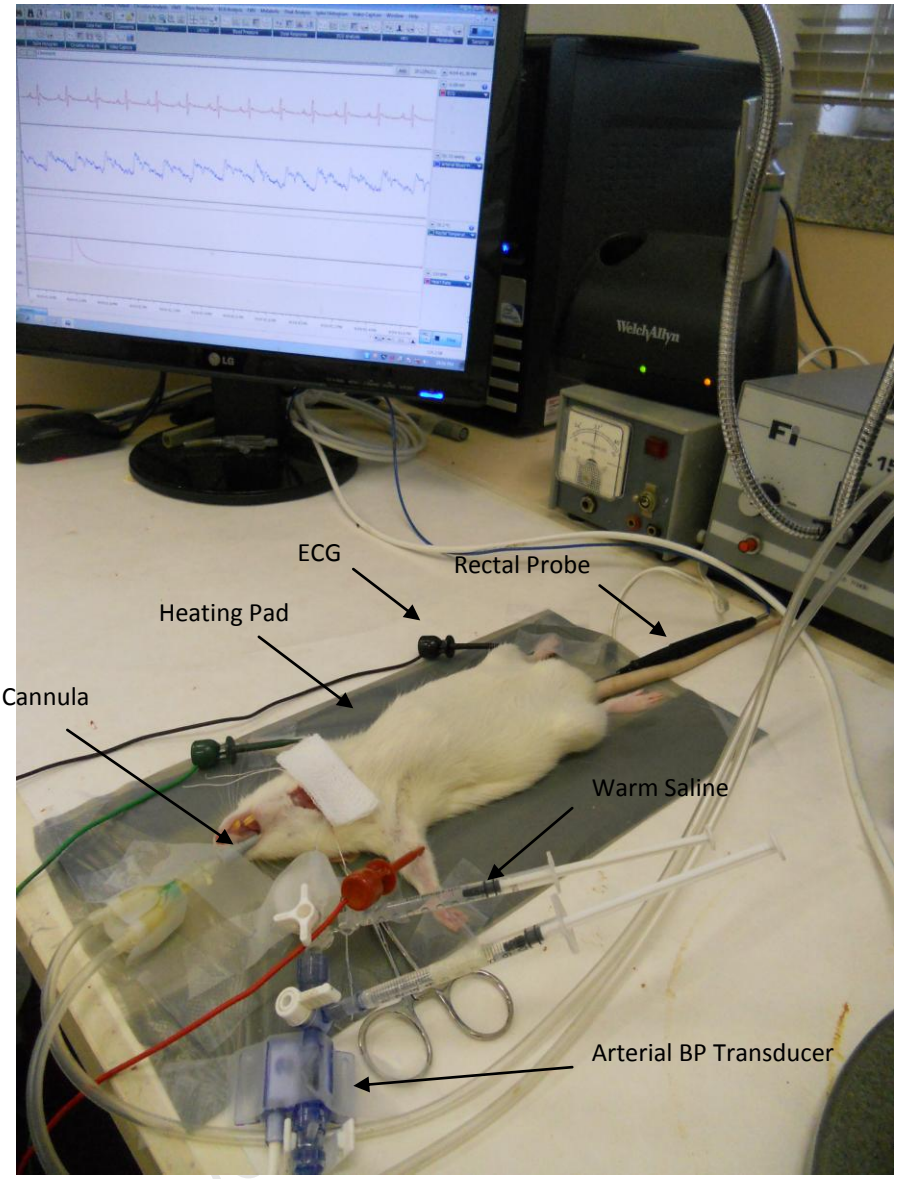


Figure 9: An example of the set-up used to assess *in vivo* functional measurements from magnesium treated rats in isoprenaline-induced myocardial infarction.

3.8 Tissue Harvesting and Gross Structural Measurements

After ECG and BP recordings, a thoracotomy was performed and the heart was excised. For the MI experiments, hearts were flushed with cold saline (4°C) through the aorta, weighed (DeltaRange, Mettler Instruments, SUI) and stored at -20°C for chemical staining and analysis. To avoid freeze damage of the epicardium, hearts were wrapped in generic cling wrap before being frozen. Blood was also collected at exsanguination and plasma was obtained by centrifuging the blood at 2000g for 15mins (Eppendorf Geratebau, Netheler Hinz, DEU). The plasma was snap frozen in liquid nitrogen and stored at -80°C for lipid

peroxidation studies. For the hypertrophy experiment, hearts were prepared for cryosectioning. The heart was excised after ECG and BP recordings, flushed with chilled saline, perfused with optimal cutting temperature (OCT) (SMM Instruments, RSA) and weighed. Hearts were then placed in a cylinder and snap frozen in liquid nitrogen before being stored at -80°C. Organs such as the lungs, liver, kidneys and adrenal glands were inspected, excised and weighed.

3.9 Quantification of Infarct Size with TTC Staining

Quantification of infarction by triphenyltetrazolium chloride (TTC) was conducted only in the MI model. The left ventricle was hand-cut transversely into 2mm thick slices. The slices were incubated in sodium phosphate buffered 1% TTC solution (pH 7.4) for 20 mins at 37^{o}C in the dark (agitated after 10mins) and were subsequently stored in 10% formalin solution in a dark cupboard at room temperature. Slices were placed on glass slides and digitally scanned 24 hours later. The infarct size was measured as the region of interest using ImageJ software (Version 1.44p, National Institute of Health, USA) (See Appendix 9.2). In a TTC-positive reaction, viable tissue turns brick red in colour due to the reaction with LDH (Sharma et al., 2001), whereas necrotic tissue that experiences LDH leakage appears pale (TTC-negative reaction). Infarct size was quantified as the percentage of TTC-negative area to total ventricular area. TTC has been known to elicit very similar results in measuring of infarct size as compared to echocardiography (dos Santos et al., 2008).

3.10 Haematoxylin and Eosin Staining

3.10.1 Cryosectioning of Frozen Tissue

Tissue was prepared for staining with Haematoxylin and Eosin (H&E) by cryosectioning. Cryosectioning the heart was conducted only in the hypertrophy model. The entire heart was frozen in OCT compound, and attached to the cryostat (CM1850, Leica Microsystems GmbH, DEU). Excess OCT was shaved away and 8 µm sections were sliced from the apex and mounted onto normal frosted end glass slides. Sections were taken sequentially through the entire ventricle. The first section was sliced at 8 µm thickness and the second section was taken 50 µm from the previous section with the third section taken 50 µm from the second section. Three sections made up one slide and five slides were taken for each heart. Each

slide's first section was separated from the previous slide's last section by 500 µm (Fig. 10). This method ensured that the entire ventricular portion was sectioned in all hearts, including the hypertrophied hearts.

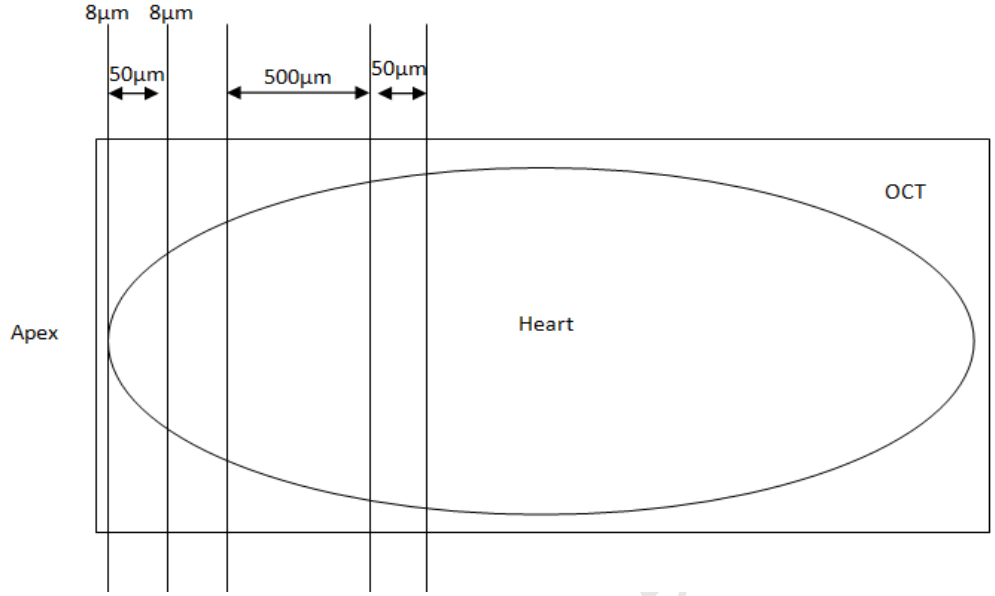


Figure 10: Schematic representation of the distance between sections used to cryosection heart tissue.

3.10.2 Haematoxylin and Eosin Staining Procedure

H&E staining was conducted on all cryosectioned hearts from the hypertrophy model to visualise tissue architecture and fibrosis (Antos et al., 2001; Ng et al., 2002). This histological stain is routinely used to assess the present state of the nuclei and the general cellular architecture. Sections were first stained with Mayer's haematoxylin (progressive stain) and then rinsed and counter-stained with eosin (See Appendix 9.3). The sections were "blued" using Scotts tap water (which has an alkaline pH) to facilitate the formation of tissue dye lakes. The sections were then rinsed and dehydrated with graded strengths of alcohol to remove excess eosin. The sections were then cleared with xylene and coverslipped with permanent mounting media (Brown, 2002). To address overstaining that was noticed in the pilot studies, we performed separate experiments to determine the optimal thickness of sections, duration of eosin staining and to evaluate the impact of post-fixation with 10% neutral buffered formalin (Fig. 11). Different section thickness of 6 µm, 8 µm and 10 µm, and different durations of eosin staining of 30s, 60s and 90s were used in post-fixed and

non-fixed tissue. We found that a section thickness of 8 µm incubated in eosin for 30s produced optimal staining in tissues that were not post-fixed.

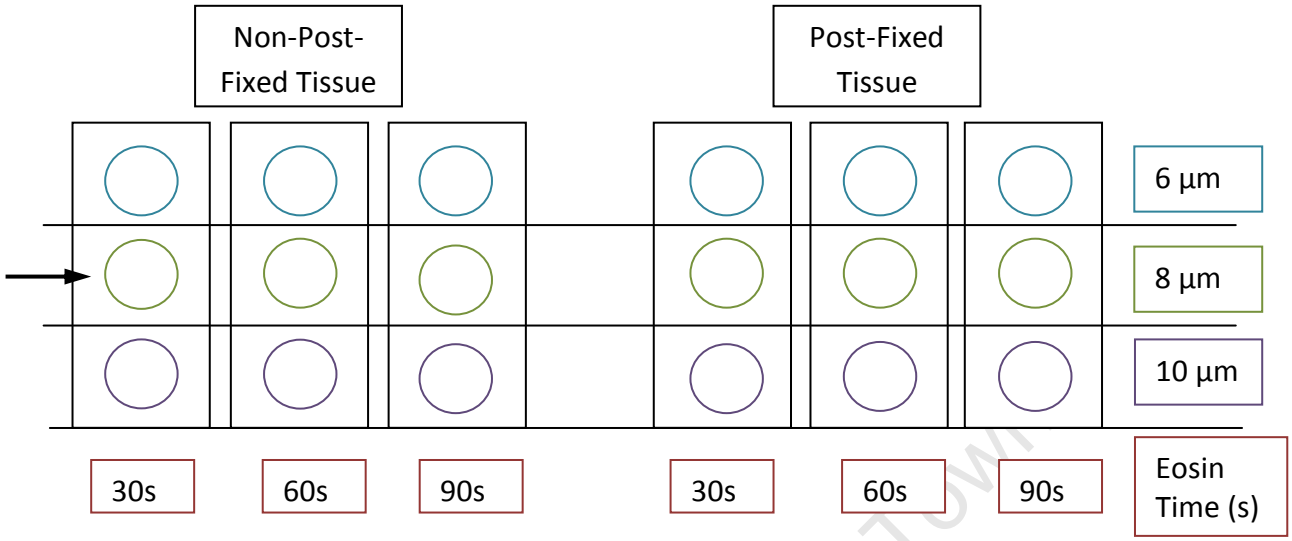


Figure 11: The experimental design for optimisation of the H&E protocol. Sections were either non-post-fixed or post-fixed and sliced at thicknesses of 6 µm (blue), 8 µm (green) and 10 µm (purple). The sections were incubated in eosin for 30s, 60s or 90s (red). The arrow points to the optimal method.

3.10.3 Quantification of Necrosis

Mounted tissue sections (8 µm) were viewed in an upright widefield microscope (AxioSkop 200, Zeiss, DEU) with transmitted light using a 5x objective. Images were captured with a colour, digital, charged-coupled device camera (AxioCam HRc, Zeiss, DEU) using AxioVision 4.7 software (Zeiss, DEU). Scale bars indicate final magnification size. The microscope magnification was 10x (eyepieces) X 5x objective (See Appendix 9.11). The resolution of the microscope is approximately 0.2 µm and clear visibility of structures in thick sections is restricted due to limited penetration depth. After microscopy, the micrographs were analysed digitally.

The level of necrosis was quantified according to the method described by van Putten et al. (2010), whereby the H&E areas can be quantified separately after staining by using a colour deconvolution plugin from ImageJ (http://rsb.info.nih.gov/ij/). This plugin enables the

quantification of fibrotic/necrotic area and the "healthy area" by separating the green, blue and red components using a built-in stain vector (Ruifrok et al., 2001). The quantification of fibrosis followed the equation: (whole area – healthy area) / whole area. When the algorithms contained in the plugin were applied to the cryosectioned, H&E stained hearts; we noticed that they were not accurate. Therefore an algorithm specific to our samples was created. We micrographed sections of hearts that were stained separately with either haematoxylin or eosin. We then used the protocol described by G Landini (http://www.dentistry.bham.ac.uk/landinig/software/cdeconvodeconv.html) to create an algorithm that allowed for accurate colour deconvolution of the H&E stained specimens. Background correction was performed with picture editing software (Photo Studio 5, ArcSoft, USA). Images were transferred to ImageJ for further background subtraction and quantification of the necrotic and fibrotic areas, connective tissue and recently regenerated tissue. The whole area of the cells was determined on the original picture and the quantification of the healthy area was determined from the red vector of the colour deconvolution tool. The threshold was adjusted so that it was representative of the original picture and quantified (See Appendix 9.3).

3.11 Lipid Peroxidation Assays

3.11.1 Conjugated Dienes

The concentration of conjugated dienes (CD) was determined using the assay described by Esterbauer et al. (1989). Briefly, 100 µL of plasma was added to 405 µL chloroform: methanol (2:1). After centrifugation at 6000g for 15 mins, the top aqueous layer was removed and the organic layer was isolated and dried under nitrogen. Cyclohexane (0.25 ml) was added to solubilise the dry organic residue and the absorbance was read at 234 nm on a spectrophotometer (Spectramax Plus 384, Molecular Devices, Labotec, RSA) using Softmax Pro (Version 4.4) software. A molar extinction coefficient of 2.95 x 10^4/M/cm was used.

3.11.2 Thiobarbituric Acid Reactive Substances

Thiobarbituric acid reactive substances (TBARS) were measured using the method described by Jentzsch et al. (1996). Briefly, to 50 µL of plasma samples, 6.25 µL of 4 mM butylated hydroxytoluene/ethanol and 50 µL of 0.2 M ortho-phosphoric acid was added and the

samples vortexed. Thiobarbituric acid (TBA) reagent (6.25 µL), dissolved in 0.1 M sodium hydroxide, was added and the mixture was microfuged at 3000g for 2mins to collect small volumes at the bottom of the Eppendorfs. The volumes were heated at 90°C for 45 mins, placed on ice for two mins and then placed at room temperature for five mins before n-butanol (500 µL) was added. Phase separation was enhanced by the addition of 50 µL of saturated sodium chloride. The samples were vortexed and centrifuged at 12 000g for two mins and 300 µL of the top butanol phase was transferred into wells and read at 532 nm on a spectrophotometer (Spectramax Plus 384, Molecular Devices, Labotec, RSA) using Softmax Pro (Version 4.4) software. A molar extinction coefficient of 1.54 x 10^5/M/cm was used. CD and TBARS measurements were performed in triplicate and the mean value was taken as the final result.

3.12 Drugs and Chemicals

Sodium pentobarbitone was purchased from Kyron Laboratories (Johannesburg, RSA). OCT was purchased from SMM Instruments (Cape Town, RSA). The rest of the drugs and chemicals were obtained from Sigma-Aldrich (Johannesburg, RSA).

3.13 Statistical Analysis

All data are expressed as mean ±standard error of the mean (SEM). All statistical analysis was conducted on Prism 5 (Graphpad, USA). A box plot analysis, whiskers: min-max, was conducted to exclude any outliers (See Appendix 9.9). Statistical tests were conducted only on the animals that were included within the box plot and survived the full 24 hour or nine day period (See Appendix 9.9). Column statistics were conducted to check if the data passed normal distribution with the Kolmogorov-Smirnov, D'Agostino and Pearson and the Shapiro-Wilk normality tests. For characterisation of the ISO model, comparisons between groups were performed using an unpaired two-tailed Student's t-test. For the Etn and Mg^{2+} studies, a One-Way ANOVA was conducted on normally distributed data followed by a Tukey post-hoc test. For the non-normally distributed data, a Kruskal-Wallis test was conducted followed by a Dunns post-hoc test. A value of $P<0.05$ was considered statistically significant.

RESULTS

4.1 Characterisation of a Low-Mortality Isoprenaline-Induced Acute Myocardial Infarction Model

4.1.1 Pilot Tests to Identify an Optimum Dose of Isoprenaline to Induce Infarction

ISO has long been used to pharmacologically induce MI in rats. However, the optimal dose of ISO required to produce a significant infarction is uncertain as not all the mortality rates are reported and high doses of ISO coincide with a high mortality rate. A pilot study was therefore conducted to identify the dose of ISO which resulted in a quantifiable infarct size but a low mortality rate. ISO was injected subcutaneously at different doses and different dilution ratios. The results (Table 2) indicated that the dose and dilution of ISO used by Arteaga de Murphy et al. (2002), whereby ISO is diluted at 20 mg/ml and injected subcutaneously at 67 mg/kg, provided an acceptable mortality rate.

Table 2: Isoprenaline injected at different doses and dilution ratios in order to optimise the dose of isoprenaline for our studies.

Number of rats injected	Dilution ratio of ISO in saline (mg/ml)	Dose of ISO injected (mg/kg)	Number of rats died
3	100	100	3
2	20	100	2
3	20	67	0

4.1.2 Isoprenaline Induces a Significant Infarction

Figure 12A shows examples of TTC-stained cross-sections of ventricular myocardium 24 hours after pre-treatment with either saline (control) or ISO (67 mg/kg, diseased). The saline-treated myocardium was positive for TTC (brick red colour) with smaller background TTC-negative areas (pale colour), whereas the diseased myocardium had a larger TTC-negative area, indicating the presence of infarction. Figure 12B shows the summary results of infarct size as determined by TTC staining. The control rats exhibited a background TTC-

negative artefact of 24% ±2%. The diseased rats displayed a TTC-negative area of 64 ±3% (P<0.001 vs. control).

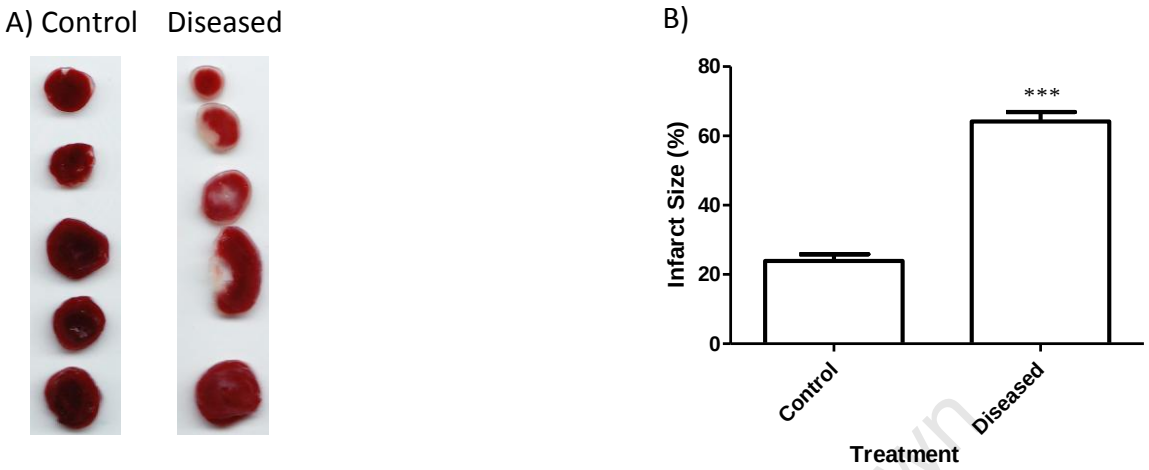


Figure 12: Isoprenaline-induced acute myocardial infarction. A) Images of ventricular myocardium cross-sections visualised with TTC staining in rats pre-treated with saline (left panel) and isoprenaline (right panel). B) Summary data of infarct size. Infarct size is expressed as a percentage of the TTC-negative area to total ventricular area. ***P<0.001 (treatment vs. control).

4.1.3 Isoprenaline Elicits a Moderate Mortality Rate

There were no deaths of rats in our control group. The death rate in the diseased group was 23% (6 out of 26 animals died) within 24 hours. Most of the deaths occurred in the first few hours after ISO injection. The low mortality rate enabled us to use the MI model for further characterisation. Soon after the ISO injection, most rats became inactive, experienced respiratory distress and had some facial stains of mucosal bleeding before recovering. All results include only animals that survived the full 24 hour period (18 control and 20 diseased animals).

4.1.4 Isoprenaline Affects both Cardiac and Other Non-cardiac Structures

Table 3 shows the ISO-induced changes in organ weight and BW. Diseased rats experienced a significant loss of BW 24 hours after treatment compared to control rats (P<0.001 vs. control). The diseased rats also had an increased HW/BW ratio compared to control rats (P<0.001). The liver/BW ratio of diseased rats was significantly lower than control rats

(P<0.01). ISO neither affected the gross structural appearance of the lungs, kidneys and adrenal glands nor the relative weights of these organs. Visual inspection of the pleural or peritoneal cavities showed no fluid accumulation as would be seen in congestive cardiac failure.

Table 3: Changes in organ and body weight in control and diseased animals. Organ weight values are shown relative to body weight.

Characteristic	Control	Diseased
BW lost (%)	-0.18 ± 0.53	-4.02 ± 0.35***
HW/BW ratio	3.45 ± 0.03	4.68 ± 0.06***
Liver/BW ratio	51.61 ± 1.02	46.23 ± 1.17**
Kidney/BW ratio	9.23 ± 0.49	8.65 ± 0.42
Adrenal/BW ratio	0.31 ± 0.02	0.33 ± 0.02
Lung/BW ratio	5.95 ± 0.45	5.18 ± 0.34

**P<0.01, ***P<0.001 (treatment vs. control)

4.1.5 Effects of Isoprenaline on Cardiac Electrophysiology

Figure 13 shows the typical lead II ECG tracings accompanying the control and diseased rats. In general, ISO treatment produced low-voltage ECG recordings (Fig. 13B). Table 4 summarises the ECG data. ISO did not alter the heart rate or the duration of the QTc and PR intervals. The drug decreased the amplitude of the P-wave (P<0.01), R-wave (P<0.001) and S-wave (P<0.01) compared to control rats. In contrast, ISO induced pathologically large Q-waves (P<0.01), an effect consistent with an evolving MI.

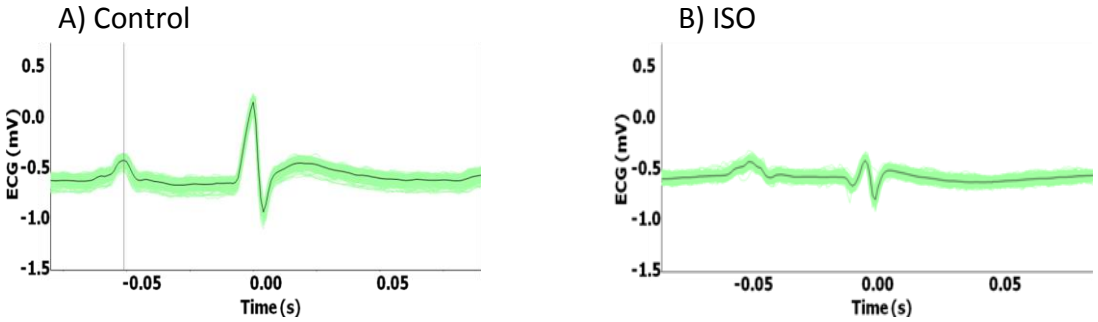


Figure 13: Superimposed original lead II ECG tracings from all saline-treated (control, A) and isoprenaline-treated (diseased, B) rats. Traces from individual rats are shown in green and the average trace for each treatment group is shown in black, n=18 for control rats and n=26 for isoprenaline-treated rats.

Table 4: Summary data of the ECG parameters.

ECG Characteristic	Control	Diseased
Heart Rate (bpm)	413.00 ± 6.84	411.00 ± 8.22
QTc (s)	0.16 ± 0.01	0.13 ± 0.01
PR Interval (s)	0.049 ± 0.002	0.048 ± 0.002
P-amplitude (mV)	0.19 ± 0.01	0.12 ± 0.02**
Q-amplitude (mV)	-0.038 ± 0.004	-0.128 ± 0.021**
R-amplitude (mV)	0.62 ± 0.03	0.17 ± 0.01***
S-amplitude (mV)	-0.30 ± 0.05	-0.12 ± 0.03**
T-amplitude (mV)	0.14 ± 0.01	0.04 ± 0.02***

**P<0.01, ***P<0.001 (treatment vs. control)

4.1.6 Isoprenaline Causes Arterial and Left Ventricular Hypotension

Figure 14 shows the ISO-induced changes in carotid artery BPs. Rats treated with ISO had decreased arterial systolic BPs compared to control rats (117 ±3.3 mmHg vs. 151 ±4.3mmHg; P<0.001). Similarly, ISO also decreased both the diastolic and dicrotic notch BPs (diastolic pressure: 94 ±4.1 mmHg vs. 122 ±2.9 mmHg; P<0.001 vs. control; dicrotic pressure: 101 ±4.3 mmHg vs. 132 ±3.2 mmHg; P<0.001 vs. control). As shown in Table 5, ISO decreased left ventricular maximum pressure (P<0.05 vs. control), the minimum rate of left ventricular pressure decline (dP/dt min) (P<0.01 vs. control) and the maximum rate of left ventricular

contraction (dP/dt max) (P<0.01 vs. control). The systolic duration was also decreased (P<0.01 vs. control), but the diastolic duration was unaffected.

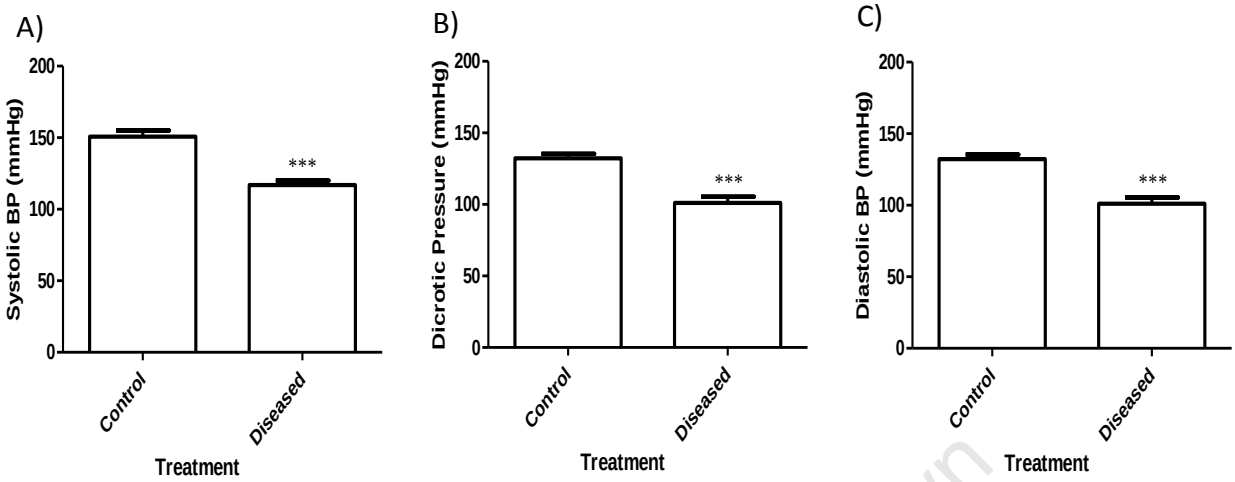


Figure 14: Carotid arterial systolic (A), dicrotic notch (B) and diastolic (C) blood pressures in control and isoprenaline-treated rats. *P<0.001 (treatment vs. control).

Table 5. Summary of *in vivo* left ventricular pressures affected by isoprenaline.

Left Ventricular Pressure Parameter	Control	Diseased
Max Pressure (mmHg)	121.5 ± 4.9	105.4 ± 4.2*
Min dP/dt (mmHg/s)	-8016 ± 587	-5679 ± 269**
Max dP/dt (mmHg/s)	8411 ± 747	6308 ± 177**
Systolic Duration (s)	0.075 ± 0.002	0.069 ± 0.001**
Diastolic Duration (s)	0.071 ± 0.002	0.076 ± 0.004

*P<0.05, **P<0.01 (treatment vs. control)

4.1.7 Isoprenaline Causes an Increase in Oxidative Stress

To determine the effects of ISO on oxidative stress, the products of lipid peroxidation (CD and TBARS) were assayed. The CD in the plasma of control rats (Fig. 15A) was lower compared to the ISO treated rats (39.87 ±3.96 µmol/L vs. 59.56 ±2.63 µmol/L; P<0.001). Measurement of TBARS (Fig. 15B) 24 hours after ISO-induced MI showed no significant difference between ISO and control rats (4.88 ±0.40 µmol/L vs. 4.10 ±0.17 µmol/L; P=0.068) although there was a trend for the expression of TBARS in ISO treated rats to drop below basal levels.

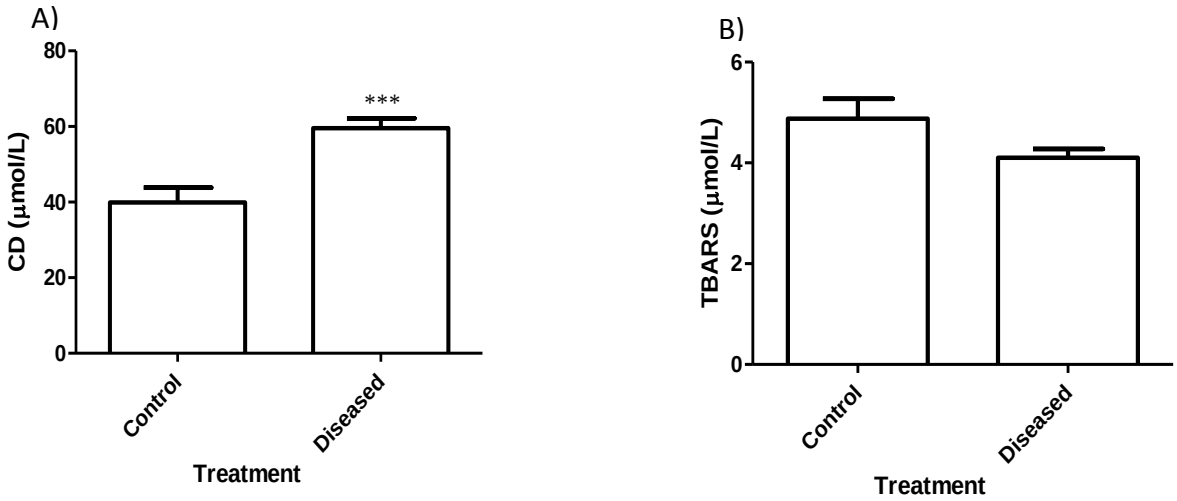


Figure 15: Effects of isoprenaline on products of lipid peroxidation; CD (A) and TBARS (B), in rat plasma. ***P<0.001 (treatment vs. control).

4.2 Effects of Ethanolamine

4.2.1 Dose-Response Curve for Ethanolamine

Previous research had focused on the effects of acute Etn administration *ex vivo*, where the hearts were perfused by the Langendorff technique and pre-treated with Etn at 0.1, 0.3, 1.0 or 5.0 mmol/L (Kelly et al., 2010). Kelly et al. (2010) state that *ex vivo*, a concentration of 0.3 mmol/L was cardioprotective. The dose used *ex vivo* was perfused directly into the heart. When administering a drug *in vivo*, a dose-response has to be established. Therefore a dose-response was carried out to determine the optimal dose and timing of Etn administered *in vivo*. To determine the optimal dose, rats (n=10) were injected intraperitoneally with Etn at 0, 5, 10 and 20 mg/kg (n=2 rats per group). Pre-treatment with Etn at 5 and 10 mg/kg appeared to lessen the ISO-induced infarct size (Fig. 16). Administration of ISO caused severe disruptions to the electrophysiology of the heart (low-voltage recordings with pathologically large Q-waves), pre-treatment with Etn (10 mg/kg) appeared to restore the disruptions back to control levels (Fig. 17). There were no differences in blood glucose, liver/BW ratio, HW/BW ratio and the percentage of BW lost between the different treatments of Etn. A dose of 10 mg/kg of Etn was identified as the optimal therapeutic dose. This dose appeared high enough to induce alterations in ECG and infarct size data, but was also low enough so as to not add any pathological disturbance to the ISO-induced MI model.

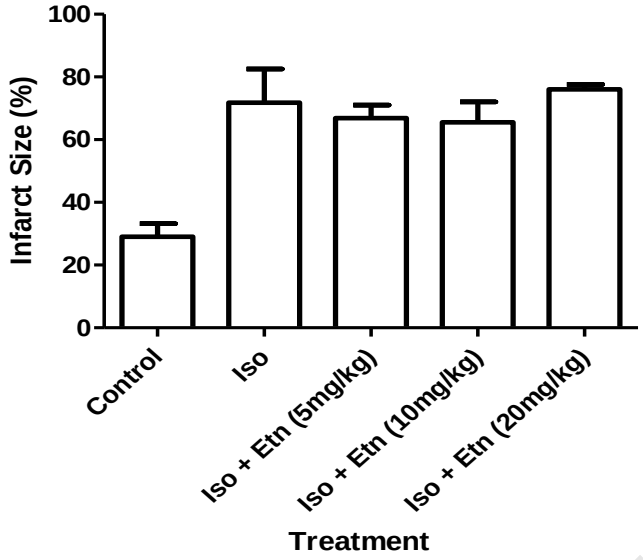


Figure 16: Summary data of infarct size. Infarct size is expressed as a percentage of the TTC-negative area to total ventricular area.

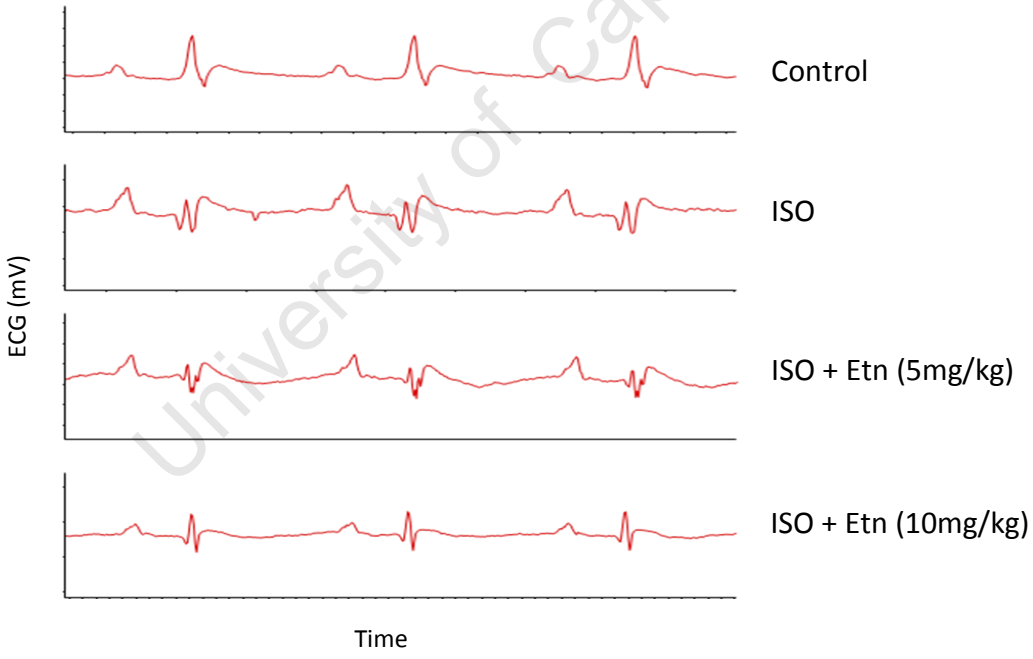


Figure 17: Representative lead II ECG tracings from rats treated with saline, isoprenaline, isoprenaline + Ethanolamine (5 mg/kg) and isoprenaline + Ethanolamine (10 mg/kg).

4.2.2 The Effects of Ethanolamine on Isoprenaline-Induced Myocardial Infarction

Treatment with ISO + Etn caused a decrease in mortality rate, but had no effect on infarct size. ISO administration disrupted the electrical activity of the heart and caused arterial hypotension. Whether treatment with Etn in a diseased rat improved electrical activity is unclear, but treatment with Etn did not improve the hypotensive state of the rat. Treatment with ISO + Etn caused an augmentation of the loss of BW and increased the HW/BW ratio compared to ISO-only treated rats. Etn may protect the lungs from ISO-induced injury.

4.2.2.1 Ethanolamine Decreases Isoprenaline-Induced Mortality

There were no deaths of rats in the control group or in the group treated with Etn alone. The model used in this study (67 mg/kg) to induce MI was associated with a low mortality with 6 deaths out of 20 rats. Furthermore, rats that received Etn pre-treatment displayed a lower mortality rate of 1 death out of 14 rats injected (Fig. 18). Most of the deaths occurred in the first few hours after ISO injection. All results include only animals that survived the full 24 hour period (n=46).

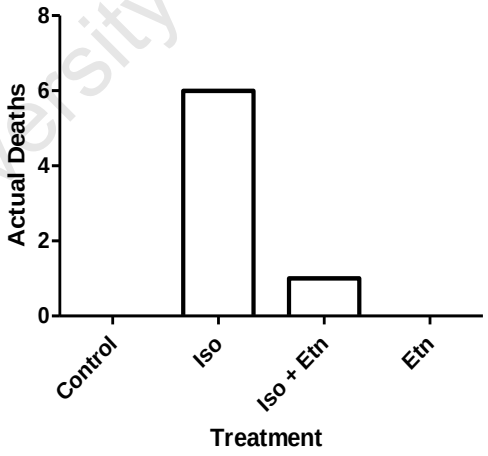


Figure 18: Number of deaths of rats receiving various treatments. Notice that pre-treatment with ethanolamine decreased the number of deaths due to isoprenaline.

4.2.2.2 Ethanolamine does not Reduce Isoprenaline-Induced Infarct Size

Figure 19A shows examples of TTC-stained cross-sections of ventricular myocardium 24 hours after pre-treatment with either saline (control), ISO (diseased), ISO + Etn or Etn alone. Figure 19B shows the summary results of infarct size as determined by TTC staining. Control rats had an infarct of 23.86 ±2.44%, this is mainly due to the background TTC-negative artefact. ISO-treated rats displayed an infarct of 61.97 ±3.85% (P<0.001 vs. control). Treatment with ISO + Etn elicited an infarct of 68.43 ±5.59% (P<0.001 vs. control). Rats treated with Etn alone had a background artefact of 17.16 ±2.30%. The TTC data were also anaysed by a second observer, using a slightly different method (See Appendix 9.2) and the same pattern of results was obtained. Control rats displayed a background artefact of 5.7 ±0.6%, ISO-treated rats elicited an infarct of 14.7 ±1.9%, rats treated with ISO + Etn had an infarct of 13.5 ±1.6% and rats treated with Etn alone had a background artefact of 5.8 ±1.0%. As the pattern of results was similar between the two methods, all other TTC data were analysed using the initial method (See Appendix 9.2).

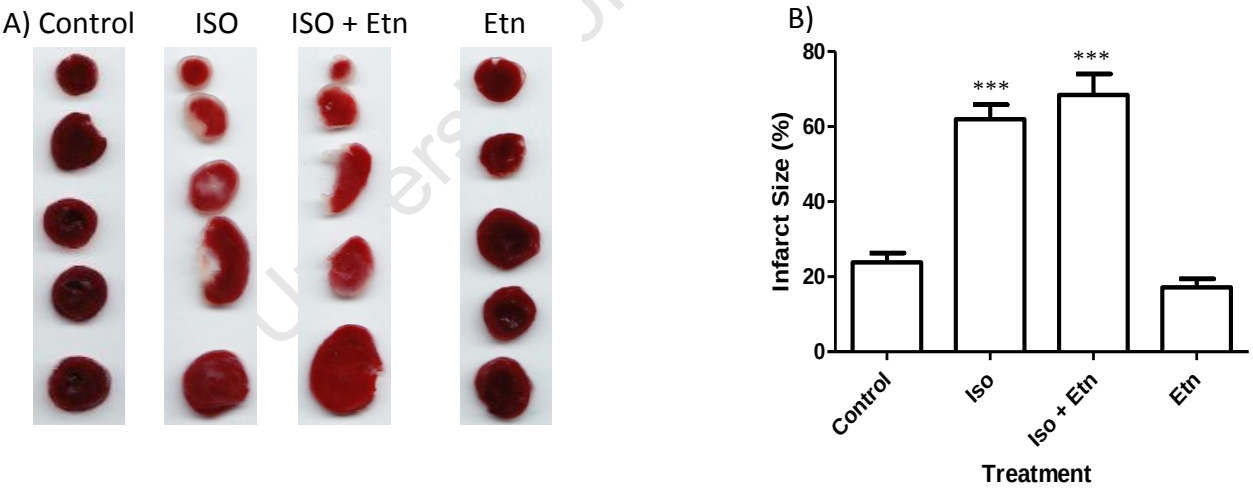


Figure 19: Isoprenaline-induced acute myocardial infarction and the effects of ethanolamine pre-treatment. A) Cross-sections of ventricular myocardium stained with TTC for visualisation of infarct size in rats pre-treated with saline, isoprenaline, isoprenaline + ethanolamine and ethanolamine alone. B) Summary data of infarct size. Infarct size is expressed as a percentage of the TTC-negative area to total ventricular area. ***P<0.001 (treatment vs. control).

4.2.2.3 Pre-Treatment with Ethanolamine Modulates the Electrical Activity of the Heart

The electrical activity of the myocardium was assessed using a 3-lead ECG and analysing lead II data in anaesthetised rats. Figure 20 shows the average lead II ECG tracings accompanying the control (A), ISO (B), ISO + Etn (C) and Etn rats (D). Table 6 summarises the quantification of the ECG data for each treatment. The heart rate, PR interval, QRS interval, QT interval and P duration were unaffected by ISO or Etn treatment. When the QT interval was corrected for heart rate using the Bazett's formula, it was found that treatment with ISO + Etn resulted in a decreased QTc compared to control rats ($P<0.05$ vs. control). This change was not observed in rats treated with ISO alone.

In general, ISO-treated rats produced low-voltage ECG recordings as seen by decreased amplitudes of the R and T-waves ($P<0.001$ vs. control). Pre-treatment with Etn in ISO-treated rats did not restore the amplitude of the R-wave ($P<0.001$ vs. control) but did increase the amplitude of the T-wave ($P<0.01$ vs. control). ISO also induced pathologically large Q-waves ($P<0.01$ vs. control), an effect consistent with an evolving MI. Rats treated with ISO + Etn exhibited normal Q-waves compared to control rats. ISO treatment caused a shortening of the T_{peak}-T_{end} parameter ($P<0.05$ vs. control). Treatment with ISO + Etn caused a further shortening of the T_{peak}-T_{end} ($P<0.001$ vs. control). Treatment with ISO + Etn appeared to decrease the S-wave and P-wave amplitudes. We noticed that the natural upslope of the ST segment in rats makes the precise identification of the J point difficult. For this reason, the ST segment was not analysed in the rats.

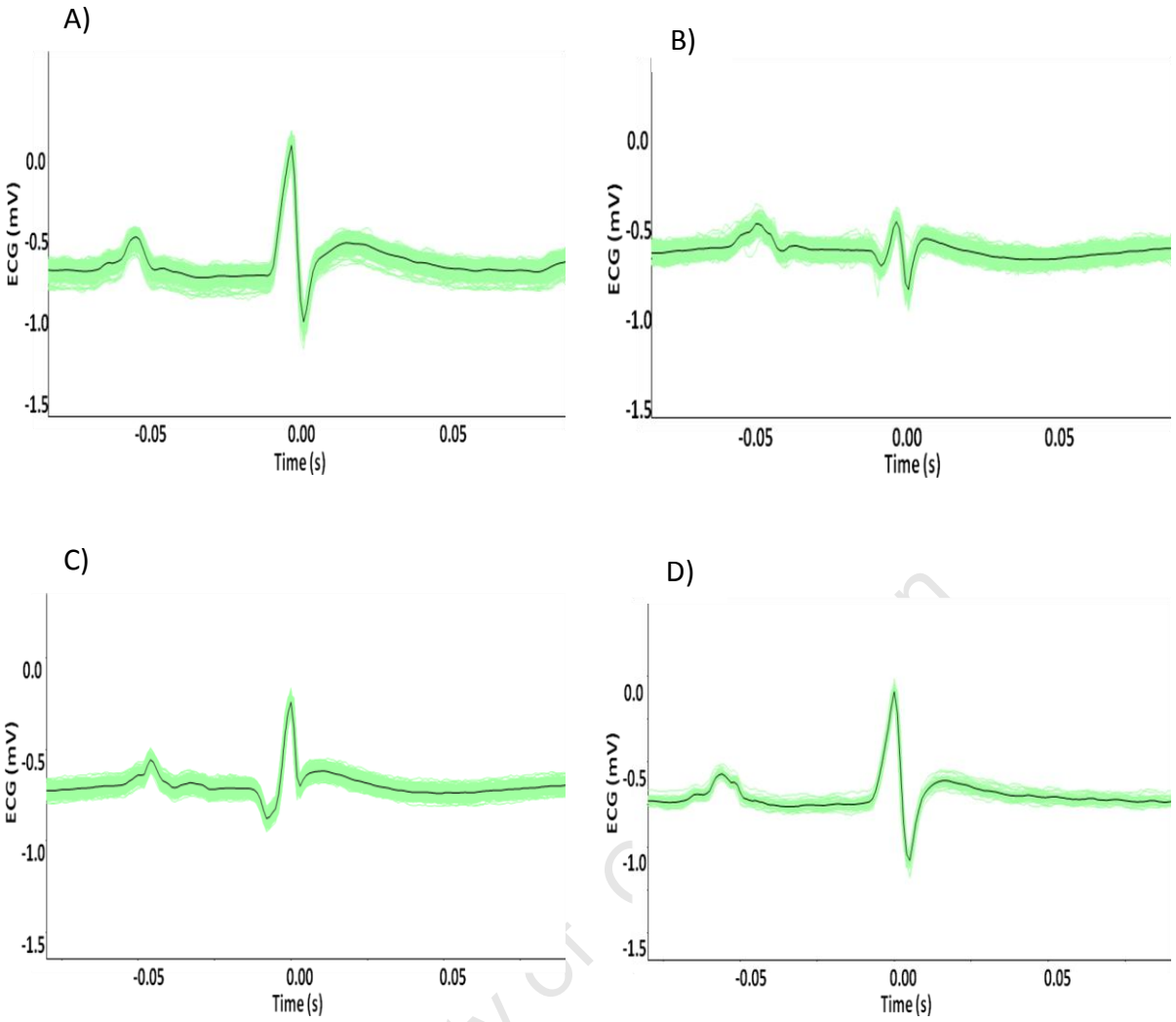


Figure 20: Effects of isoprenaline and ethanolamine on electrical activity. Superimposed original lead II ECG tracings from control (A), isoprenaline (B), isoprenaline + Ethanolamine (C) and Ethanolamine (D) rats. Dark black lines are the average tracing for n=12 rats, n=14 rats, n=13 rats and n=7 rats respectively. Green lines indicate traces from individual rats.

Table 6: Summary of ECG characteristics for the effects of ethanolamine on myocardial infarction.

ECG Characteristic	Control	ISO	ISO + Etn	Etn
Heart rate (bpm)	403.7 ± 12.4	405.8 ± 10.9	398.5 ± 7.7	394.1 ± 10.6
PR Interval (s)	0.050 ± 0.000	0.046 ± 0.002	0.046 ±0.002	0.048 ± 0.003
QRS Interval (s)	0.015 ± 0.001	0.014 ± 0.001	0.017 ± 0.002	0.016 ±0.000
QT Interval (s)	0.055 ± 0.001	0.049 ± 0.005	0.044 ± 0.005	0.056 ± 0.001
QTc (s)	0.146 ± 0.002	0.128 ± 0.014	0.100 ±0.010*	0.143 ± 0.004
T_{peak}-T_{end} (s)	0.030 ± 0.000	0.022 ± 0.002*	0.016 ± 0.002***	0.030 ± 0.000
P Duration (s)	0.018 ± 0.000	0.016 ± 0.001	0.016 ± 0.001	0.017 ± 0.001
P Amplitude (mV)	0.177 ± 0.007	0.154 ± 0.008	0.140 ± 0.009*	0.124 ± 0.031
Q Amplitude (mV)	-0.032 ± 0.008	-0.130 ± 0.268**	-0.093 ± 0.017	-0.018 ± 0.011
R Amplitude (mV)	0.648 ± 0.036	0.167 ± 0.013***	0.225 ± 0.029***	0.588 ± 0.054
S Amplitude (mV)	-0.260 ± 0.051	-0.126 ± 0.043	-0.049 ± 0.024*	-0.266 ± 0.018
T Amplitude (mV)	0.158 ± 0.014	0.044 ± 0.016***	0.054 ± 0.017**	0.120 ± 0.013

*P<0.05, **P<0.01, ***P<0.001 (treatment vs. control)

4.2.2.4 Effects of Ethanolamine on Arterial Blood Pressure

The administration of ISO caused arterial hypotension (Fig. 21) as seen by a decrease in the systolic and diastolic BP compared to control rats respectively (Control = 147.7 ±4.9 mmHg, ISO = 116.8 ±3.3 mmHg; P<0.001 and Control = 119.5 ±3.5 mmHg, ISO = 93.9 ±4.1 mmHg; P<0.01).Pre-treatment with Etn in ISO-treated rats resulted in no difference in systolic BP (113.2 ±4.6 mmHg; P<0.001 vs. control) but caused a further decrease in diastolic BP (87.6 ±4.9 mmHg; P<0.001 vs. control). ISO also caused a decrease in the dicrotic notch pressure (Control = 129.8 ±3.8 mmHg, ISO = 101.1 ±4.3 mmHg; P<0.01) and the mean diastolic pressure compared to control rats (Control = 124.9 ±3.6 mmHg, ISO = 97.5 ±3.1 mmHg; P<0.01). Pre-treatment with Etn did not rectify or worsen the dicrotic notch pressure or mean diastolic pressure respectively (96.4 ±5.8 mmHg; P<0.01 vs. control and 95.0 ±4.9 mmHg; P<0.01 vs. control).

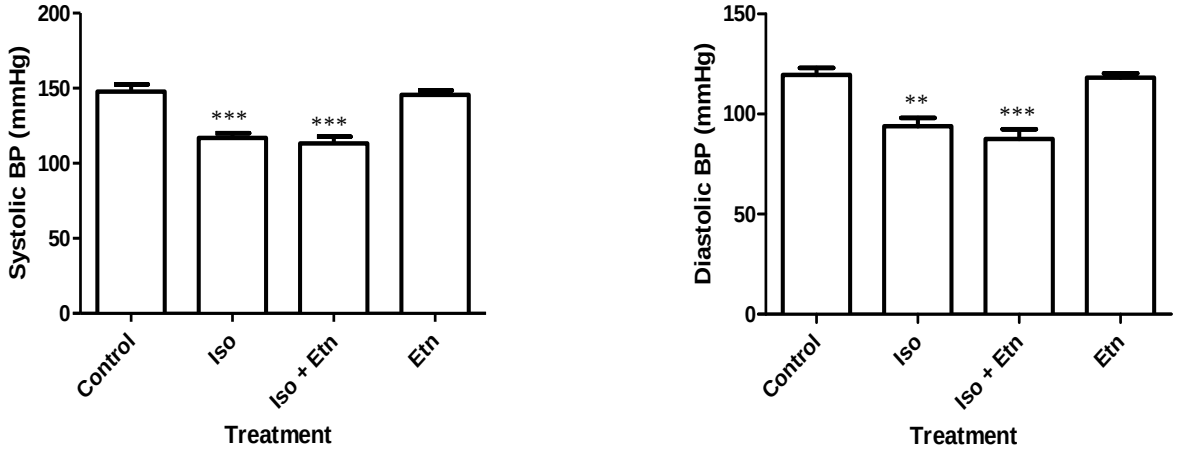


Figure 21: The effects of isoprenaline and ethanolamine on arterial blood pressure. Isoprenaline causes arterial hypotension and pre-treatment with ethanolamine does not improve this condition. **P<0.01, ***P<0.001 (treatment vs. control).

4.2.2.5 Effects of Isoprenaline and Ethanolamine on Cardiac and Non-Cardiac Structures

Table 7 highlights the effects of ISO and Etn administration on cardiac and non-cardiac structures. ISO caused a loss in BW (P<0.01 vs. control) and treatment with ISO + Etn resulted in a further loss in BW (P<0.001 vs. control). Administration of ISO caused an increase in the HW/BW ratio compared to control rats (P<0.01) and treatment with ISO + Etn also further augmented the increase in HW/BW ratio (P<0.001 vs. control). ISO + Etn appeared to protect against the ISO-induced decrease in the lungs/BW ratio. ISO neither affected the gross structural appearance of the liver, kidneys and the adrenal glands nor impacted the weight of these organs.

Table 7: Alterations in body weight and organ weights in control, isoprenaline and ethanolamine treated rats.

Characteristic	Control	ISO	ISO + Etn	Etn
BW Lost (%)	-0.01 ± 0.52	-3.53 ± 0.55**	-5.58 ± 0.87***	-0.62 ± 0.56
HW/BW ratio	3.43 ± 0.02	4.62 ± 0.08**	5.03 ± 0.17***	3.3 ± 0.04
Liver/BW ratio	50.59 ± 1.40	45.73 ± 1.41	46.74 ± 1.06	48.95 ± 1.49
Lungs/BW ratio	7.33 ± 0.12	6.44 ± 0.09**	6.93 ± 0.19	7.14 ± 0.42
Kidneys/BW ratio	10.73 ± 0.12	10.29 ± 0.14	10.44 ± 0.11	10.9 ±0.31
Adrenals/BW ratio	0.37 ± 0.01	0.39 ± 0.01	0.42 ± 0.02	0.36 ± 0.03

**P<0.01, ***P<0.001 (treatment vs. control)

4.2.2.6 Lipid Peroxidation is Unaffected by Isoprenaline and Ethanolamine Administration

Lipid peroxidation was assessed in terms of CD and TBARS. Figure 22 shows the results observed with ISO and Etn administration. Administration of ISO did not cause a significant increase in either the CD or TBARS.

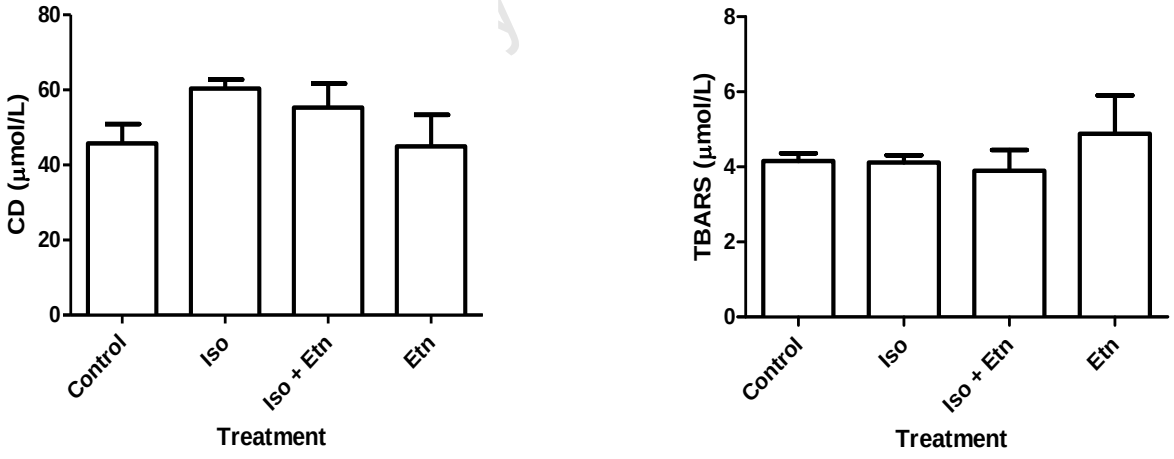


Figure 22: Effects of isoprenaline and ethanolamine on lipid peroxidation. CD and TBARS present in the rat plasma 24 hours after administration of drug interventions.

4.3 The Effects of Magnesium Pre-Treatment on Isoprenaline-Induced Myocardial Infarction

Mg^{2+} is a potent antitachydysrhythmic agent, primarily used in the treatment of electrophysiological dysfunction and hypertension. In this study, treatment with Mg^{2+} did not reduce the infarct size or prevent electrophysiological dysfunction associated with ISO administration. Mg^{2+} appeared to improve some haemodynamic parameters but could not prevent systemic disruption caused by ISO and may have negative effects on the kidneys.

4.3.1 Magnesium does not Reduce Isoprenaline-Induced Infarct Size

Administration of ISO caused an infarction to the myocardium (Fig. 23A) and the quantification of infarction is shown in Figure 23B (Control = 23.79 ±1.93%, ISO = 68.06 ±4.17%; P<0.001). Pre-treatment with Mg^{2+} did not prevent this ISO-induced infarction (62.97 ±4.12%; P<0.001 vs. control). Treatment with Mg^{2+} alone did not cause injury to the myocardium (24.37 ±2.36%).

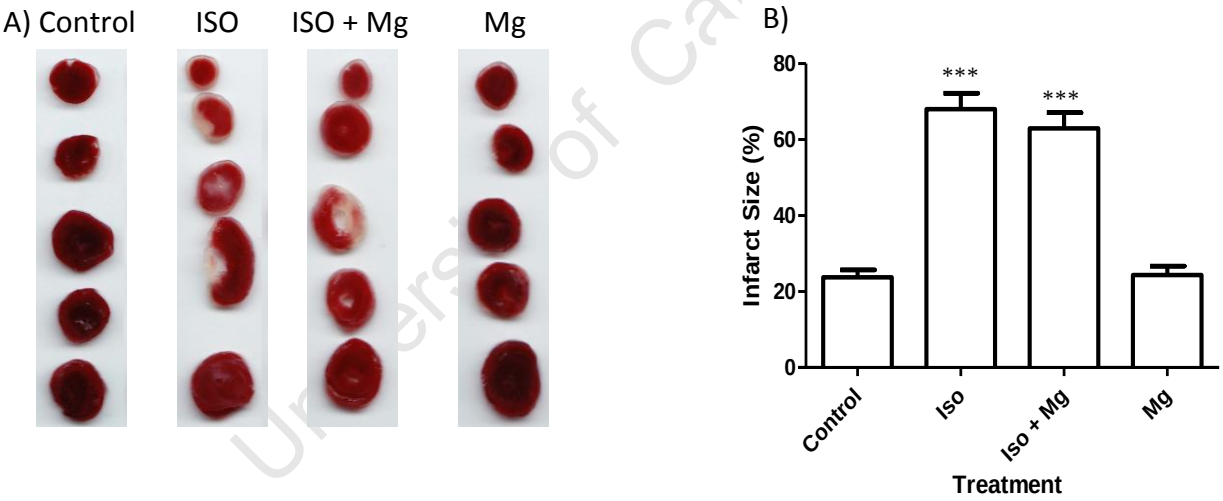


Figure 23: Isoprenaline-induced acute myocardial infarction and the effects of magnesium pre-treatment. A) Cross-sections of ventricular myocardium stained with TTC for visualisation of infarct size in rats pre-treated with saline, isoprenaline, isoprenaline + magnesium and magnesium alone. B) Summary data of infarct size. Infarct size is expressed as a percentage of the TTC-negative area to total ventricular area. ***P<0.001 (treatment vs. control).

4.3.2 Magnesium does not Prevent Isoprenaline-Induced Cardiac Electrophysiological Changes

Figure 24 shows that administration of ISO caused severe disruption to the ECG. The characteristics of the ECG are quantified in Table 8. The heart rate, PR interval, QRS interval, Qt interval, QTc interval, P duration, P amplitude and S amplitude were unaffected by administration of ISO. Mg^{2+} administered alone did not affect the ECG compared to control rats. ISO caused a decrease in the T_{peak}-T_{end} interval ($P<0.05$ vs. control) and this disruption was not rectified by administration of Mg^{2+} ($P<0.05$ vs. control). ISO affected the Q-wave ($P<0.001$ vs. control) and treatment with Mg^{2+} appeared to lessen the impact of ISO on the Q-wave ($P<0.05$ vs. control). The voltage of the ECG was lessened by ISO which is indicative of a decreased R-wave ($P<0.001$ vs. control). Treatment with ISO + Mg did not resolve this decreased voltage ($P<0.001$ vs. control). Ventricular repolarisation was disrupted when ISO was administered (T-wave=$P<0.05$ vs. control) and treatment with Mg^{2+} appeared to further disrupt this parameter ($P<0.001$ vs. control). Interestingly, ISO alone did not alter the S-wave, yet treatment with ISO + Mg caused a depressed S-wave ($P<0.05$ vs. control).

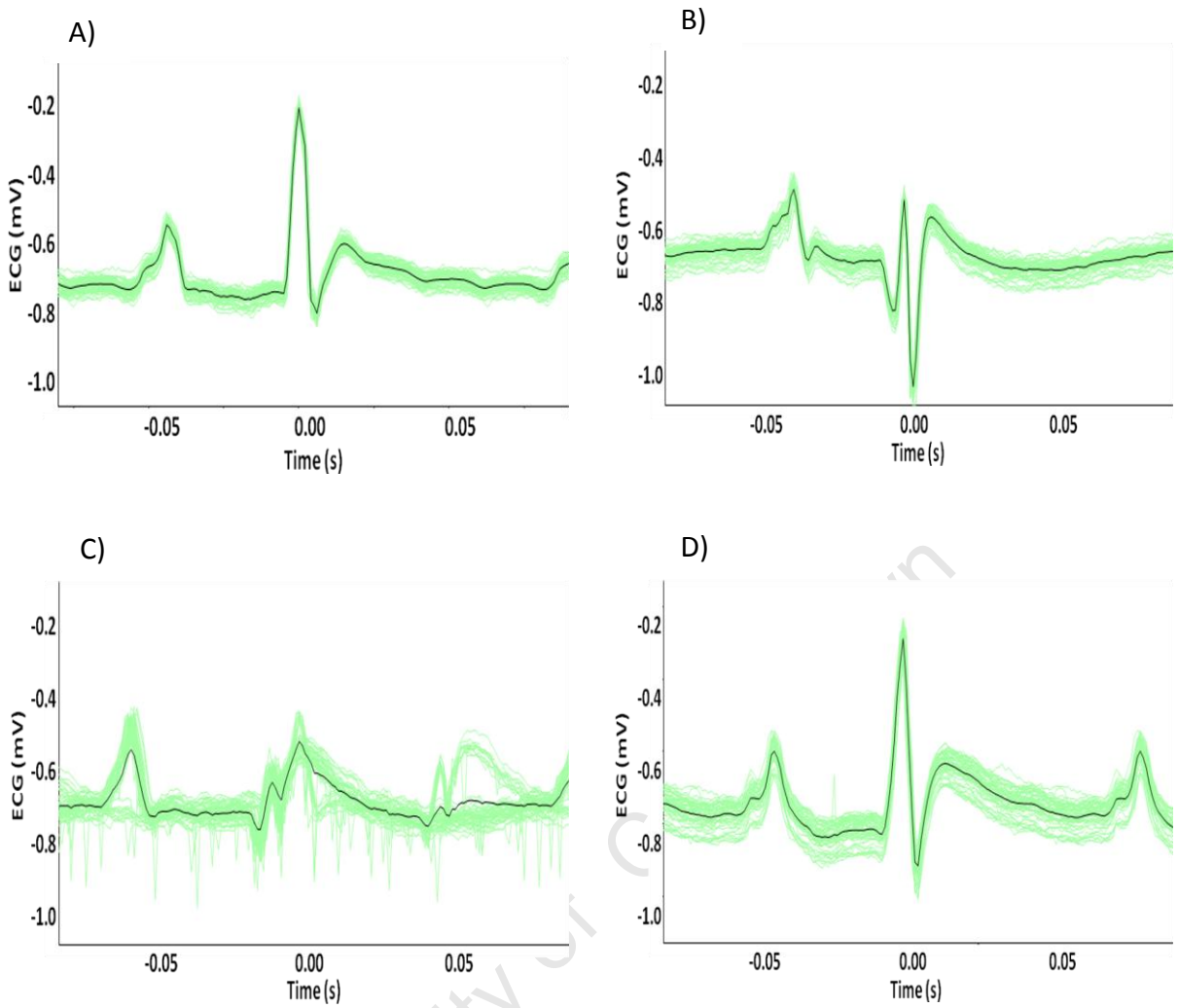


Figure 24: Effects of isoprenaline and magnesium on electrical activity. Superimposed original lead II ECG tracings from control (A), isoprenaline (B), isoprenaline + magnesium (C) and magnesium (D) rats. Dark black lines are the average tracing for n=8 rats, n=9 rats, n=10 rats and n=8 rats respectively. Green lines indicate traces from individual rats.

Table 8: Summary of the ECG characteristics for the effects of magnesium on myocardial infarction.

ECG Characteristic	Control	ISO	ISO + Mg	Mg
Heart rate (bpm)	406.9 ± 9.5	416.6 ± 14.2	418.4 ±7.2	405.8 ± 15.4
PR Interval (s)	0.046 ± 0.003	0.050 ± 0.003	0.046 ± 0.002	0.050 ± 0.002
QRS Interval (s)	0.014 ± 0.001	0.014 ± 0.001	0.013 ± 0.001	0.015 ± 0.001
QT Interval (s)	0.061 ± 0.003	0.046 ± 0.005	0.056 ± 0.007	0.054 ± 0.002
QTc (s)	0.157 ± 0.009	0.123± 0.016	0.148 ± 0.017	0.140 ± 0.004
T_{peak}-T_{end} (s)	0.040 ± 0.003	0.025 ± 0.003*	0.024 ± 0.003*	0.030 ± 0.000
P Duration (s)	0.184 ± 0.010	0.164 ± 0.009	0.166 ± 0.015	0.161 ± 0.013
P Amplitude (mV)	0.184 ± 0.010	0.165 ± 0.009	0.167 ± 0.014	0.162 ± 0.013
Q Amplitude (mV)	-0.024 ± 0.008	-0.111 ± 0.020**	-0.107 ± 0.021*	-0.025 ± 0.008
R Amplitude (mV)	0.590 ± 0.056	0.193 ± 0.030***	0.216 ± 0.031***	0.619 ± 0.044
S Amplitude (mV)	-0.300 ± 0.073	-0.129 ± 0.060	-0.050 ± 0.022*	-0.248 ± 0.054
T Amplitude (mV)	0.123 ± 0.010	0.087 ± 0.009*	0.023 ± 0.018***	0.134 ± 0.008

*P<0.05, **P<0.01, ***P<0.001 (treatment vs. control)

4.3.3 Magnesium Therapy Appears to Protect Against Isoprenaline-Induced Hypotension.

The left ventricular pressure was measured using a Mikro-tip pressure manometer. ISO did not affect the minimum pressure in the left ventricle, the end diastolic pressure, the systolic or diastolic durations, the maximum rate of pressure change (dP/dt max) or the contractility index of the left ventricle. Figure 25A shows that ISO caused a decrease in the maximum pressure in the left ventricle compared to control rats (Control=123.4 ±4.5 mmHg, ISO=101.7 ±2.2 mmHg; P<0.01). Treatment with Mg^{2+} appeared to restore this near to control levels (107.4 ±6.4 mmHg). While ISO administration did not affect the systolic duration, treatment with ISO + Mg decreased the systolic duration compared to control rats (Control=0.075 ±0.0s, ISO + Mg=0.068 ±0.0s; P<0.05). ISO decreased the minimum and average rate of pressure change in the ventricle (Fig. 26A and B) compared to control rats respectively (Control=-7921 ±434 mmHg/s, ISO=-5479 ±203 mmHg/s; P<0.001 and Control=-4354 ±158 mmHg/s, ISO=-3161 ±200 mmHg/s; P<0.01). Treatment with Mg^{2+} appeared to

improve the isovolumic relaxation of the ventricle (dP/dt min=-6040 ±491 mmHg/s; P<0.01 vs. control and dP/dt avg=-3534 ±236 mmHg/s).

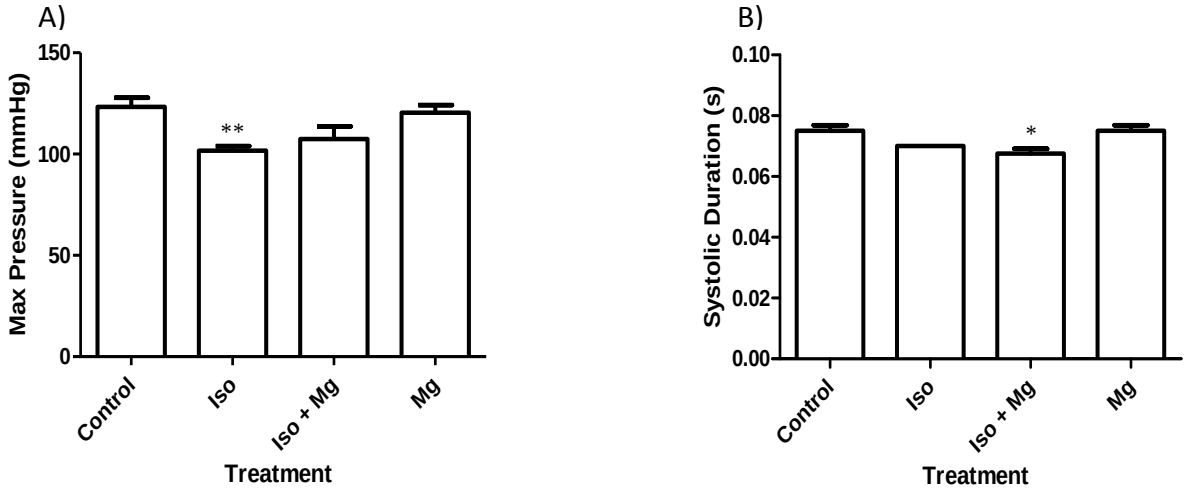


Figure 25: The maximum pressure (A) and systolic duration (B) of the left ventricle under different treatments. *P<0.05, **P<0.01 (treatment vs. control).

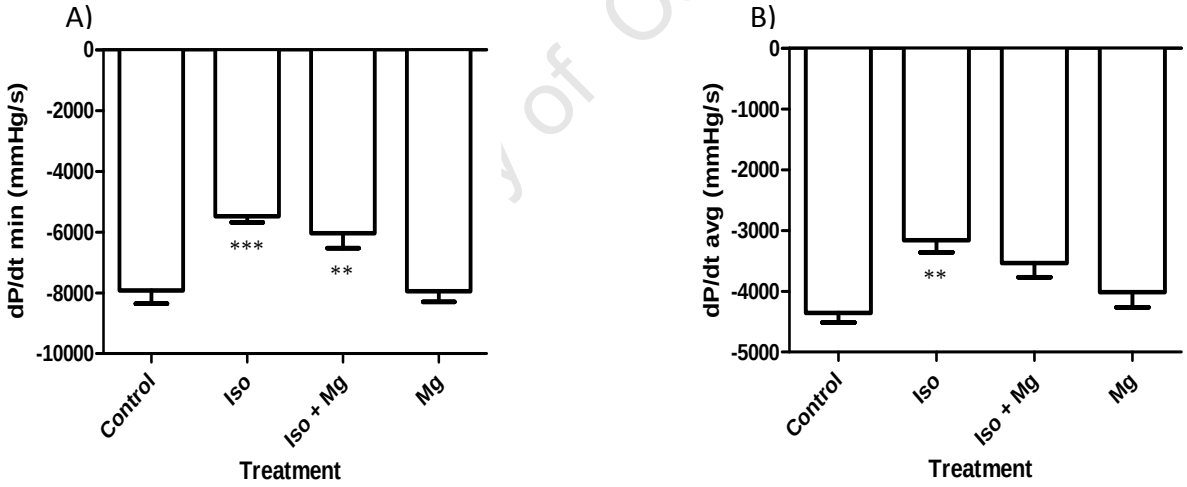


Figure 26: The minimum (A) and average (B) rate of change of pressure in the left ventricle under different treatment conditions. **P<0.01, ***P<0.001 (treatment vs. control).

4.3.4 The Effects of Isoprenaline and Magnesium on Cardiac and Non-Cardiac Structures

Table 9 shows the effects of ISO and Mg^{2+} on BW loss and organ weights. When Mg^{2+} was administered alone, it did not affect the BW lost or the weights of any organs compared to

control rats. ISO caused a loss in BW ($P<0.05$ vs. control). Treatment with Mg^{2+} did not rectify this BW loss ($P<0.05$ vs. control). ISO caused an increase in the HW/BW ratio, possibly indicating hypertrophy ($P<0.001$ vs. control). When rats were treated with ISO + Mg, the HW/BW ratio was increased to a similar extent ($P<0.001$ vs. control). ISO did not affect the gross structure or weights of the liver, lungs, kidneys or adrenal glands. Treatment with ISO + Mg caused a significant decrease in the kidney/BW ratio.

Table 9: Alterations in body weight and organ weights in control, isoprenaline and magnesium treated rats.

Characteristic	Control	ISO	ISO + Mg	Mg
BW Lost (%)	0.54 ± 0.16	-3.65 ± 0.61*	-3.77 ± 1.31*	-0.75 ± 0.80
HW/BW ratio	3.33 ± 0.06	4.82 ± 0.06***	4.74 ± 0.13***	3.34 ± 0.05
Liver/BW ratio	50.68 ± 1.11	47.34 ± 2.34	44.08 ± 1.32	50.54 ± 1.87
Lungs/BW ratio	3.62 ± 0.20	3.21 ± 0.10	3.35 ± 0.10	3.46 ± 0.20
Kidneys/BW ratio	6.86 ± 0.10	6.58 ± 0.10	6.38 ± 0.10**	6.57 ± 0.10
Adrenals/BW ratio	0.20 ± 0.00	0.24 ± 0.00	0.23 ± 0.00	0.22 ± 0.00

*P<0.05, **P<0.01, ***P<0.001 (treatment vs. control)

4.3.5 Lipid Peroxidation is Unaffected by Magnesium

After 24 hours, the levels of lipid peroxidation in the plasma of the rats were not significantly different to control values (Fig. 27A and B).

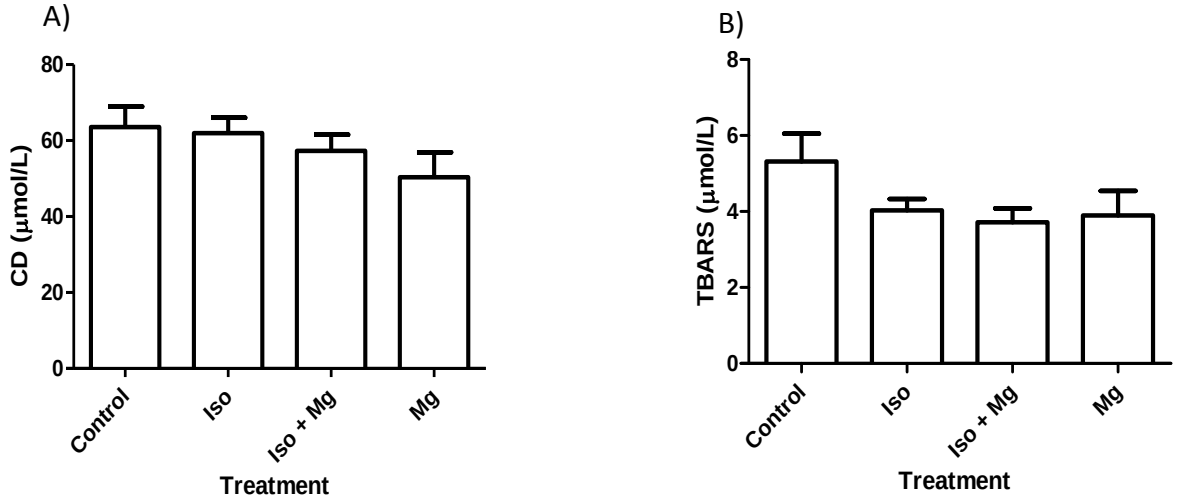


Figure 27: Effects of isoprenaline and magnesium on lipid peroxidation. CD (A) and TBARS (B) present in the rat plasma 24 hours after administration of drug interventions.

4.4 Cardiac Hypertrophy Model

4.4.1 Chronic Isoprenaline Administration Induces Cardiac Hypertrophy

Chronic administration of ISO at 5 mg/kg resulted in cardiac hypertrophy and neurological deficits. This was confirmed by histological examination shown in Figure 28, whereby ISO caused necrosis, loss of cell membrane integrity and infiltration of inflammatory cells. ISO also caused an alteration to the HW/BW ratio (Table 10). The HW/BW ratio of control rats was 4.40 ±0.0 and ISO-treated rats displayed a HW/BW ratio of 5.24 ±0.10 ($P<0.07$). There were also differences between ISO and control rats with regards to depression and anxiety states (Figs. 34-37).

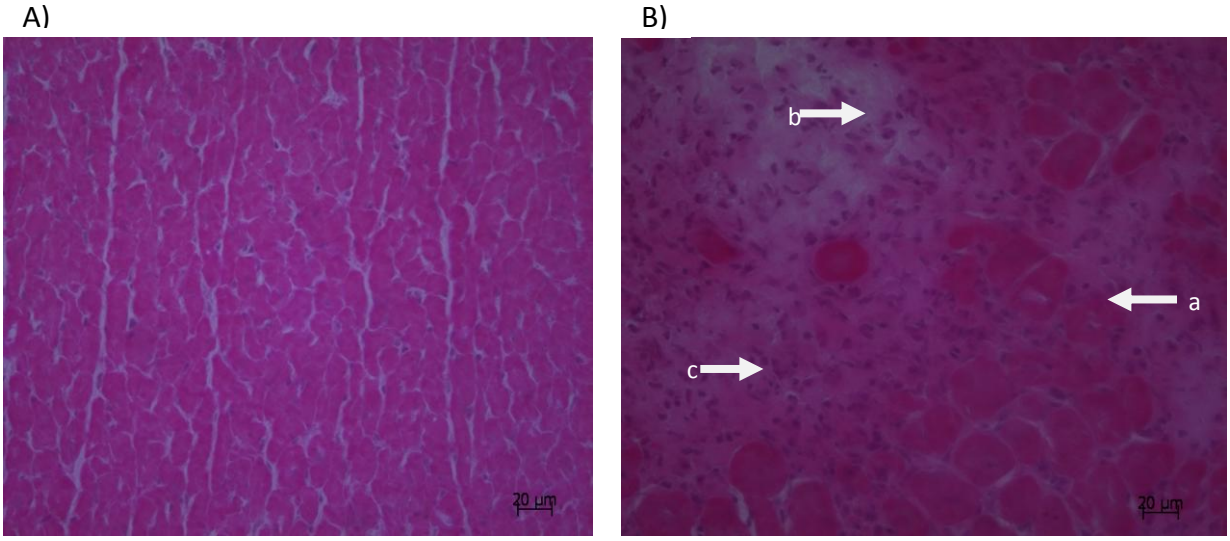


Figure 28: Micrographs of control (A) and isoprenaline-treated (B) rats H&E stained, 40X magnification. Haematoxylin stains nuclei blue and the cytoplasm and connective tissue are stained in varying shades of pink by the eosin counterstain. Myocardial cell membranes of control hearts remain in tact and there was no infiltration of inflammatory cells, unlike isoprenaline-treated hearts which show a loss of integrity of the cell membrane (a), necrosis (b) and infiltration of inflammatory cells (c).

4.4.2 The Effects of Acute Ethanolamine Administration on Isoprenaline-Induced Cardiac Hypertrophy

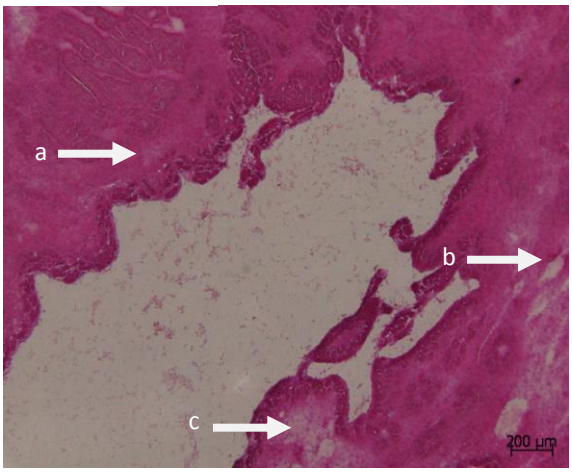
Pre-treatment with Etn appeared to protect against ISO-induced electrophysiological and neurological dysfunction. Etn in this model also caused a greater increase in the HW/BW ratio compared to ISO and control rats, and again had no impact on decreasing necrosis or fibrosis induced by ISO.

4.4.2.1 Ethanolamine does not Prevent Necrosis

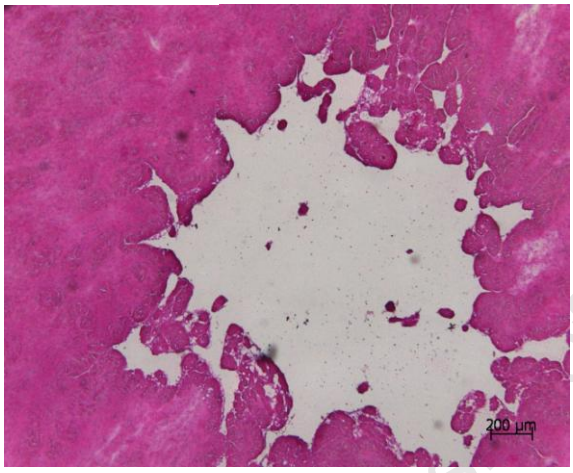
The amount of necrosis and fibrosis was assessed using H&E staining and quantification with micrographs in ImageJ. Examples of micrographs for each treatment are shown in Figure 29 with the quantification of the micrographs shown in Figure 30. ISO (Fig. 29A) caused severe necrosis of the myocardium (a), separation of cardiomyocytes (b) and an infiltration of inflammatory cells (c). The cell membrane of the saline (control) and Etn-only treated rats maintained its integrity and there was no infiltration of inflammatory cells.

A) Control

B) ISO



C) ISO + Etn



D) Etn

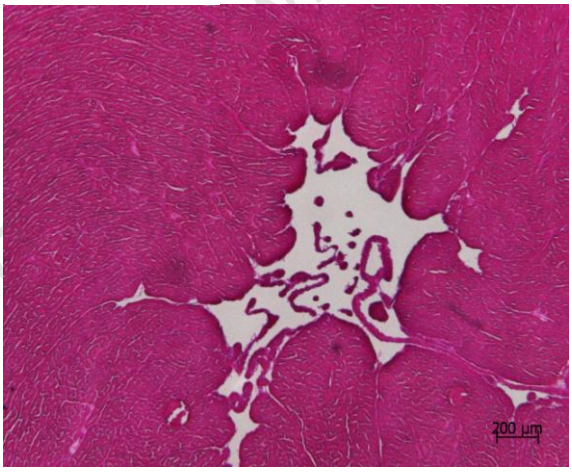


Figure 29: Sample micrographs of the different treatment groups. Isoprenaline caused severe necrosis (a), alterations in the cardiomyocyte architecture (b) and infiltration of inflammatory cells (c). Ethanolamine pre-treatment did not prevent this. H&E stained, 5X magnification.

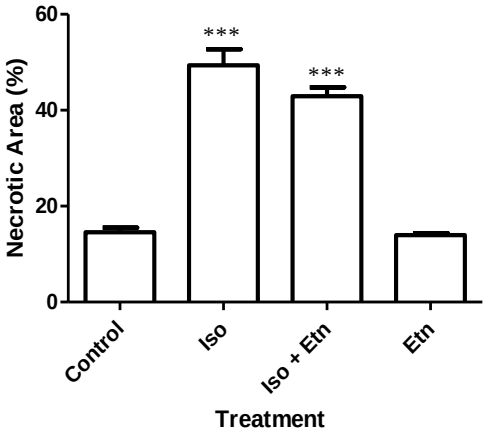


Figure 30: Quantification of the necrosis caused by isoprenaline and the effects of pre-treatment with ethanolamine. ***P<0.001 (treatment vs. control)

4.4.2.2 The Effects of Isoprenaline and Ethanolamine on Electrical Function

Table 10 shows that treatment with ISO for seven consecutive days caused a longer QRS interval (P<0.05 vs. control). Pre-treatment with Etn reduced this dysfunction back to control levels. ISO administration also caused a decrease in the voltage of the ECG as seen by a depression of the R-wave (P<0.05 vs. control). Treatment with Etn did not worsen this parameter. Interestingly, ISO did not affect the heart rate, however, treatment with ISO + Etn caused a decreased heart rate compared to control rats (P<0.01 vs. control). The combined ECG recordings for all rats per group are displayed in Figure 31.

Table 10: Summary of the ECG characteristics for the different treatment groups.

ECG Characteristic	Control	ISO	ISO + Etn	Etn
Heart rate (bpm)	389.1 ± 11.7	346.1 ± 20.9	324.5 ± 9.5**	391.0 ± 11.1
PR Interval (s)	0.051 ± 0.000	0.050 ± 0.000	0.047 ± 0.000	0.049 ± 0.000
QRS Interval (s)	0.016 ± 0.000	0.021 ± 0.000*	0.017 ± 0.000	0.015 ± 0.000
P Duration (s)	0.018 ± 0.000	0.017 ± 0.000	0.017 ± 0.000	0.017 ± 0.000
P Amplitude (mV)	0.120 ± 0.000	0.114 ± 0.100	0.089 ± 0.000	0.103 ± 0.000
Q Amplitude (mV)	-0.036 ± 0.000	-0.090 ± 0.000	-0.090 ± 0.000	-0.009 ± 0.000
R Amplitude (mV)	0.587 ± 0.000	0.401 ± 0.100*	0.405 ± 0.100	0.629 ± 0.100
S Amplitude (mV)	-0.217 ± 0.000	-0.101 ± 0.000	-0.189 ± 0.000	-0.240 ± 0.000

*P<0.05, **P<0.01 (treatment vs. control)

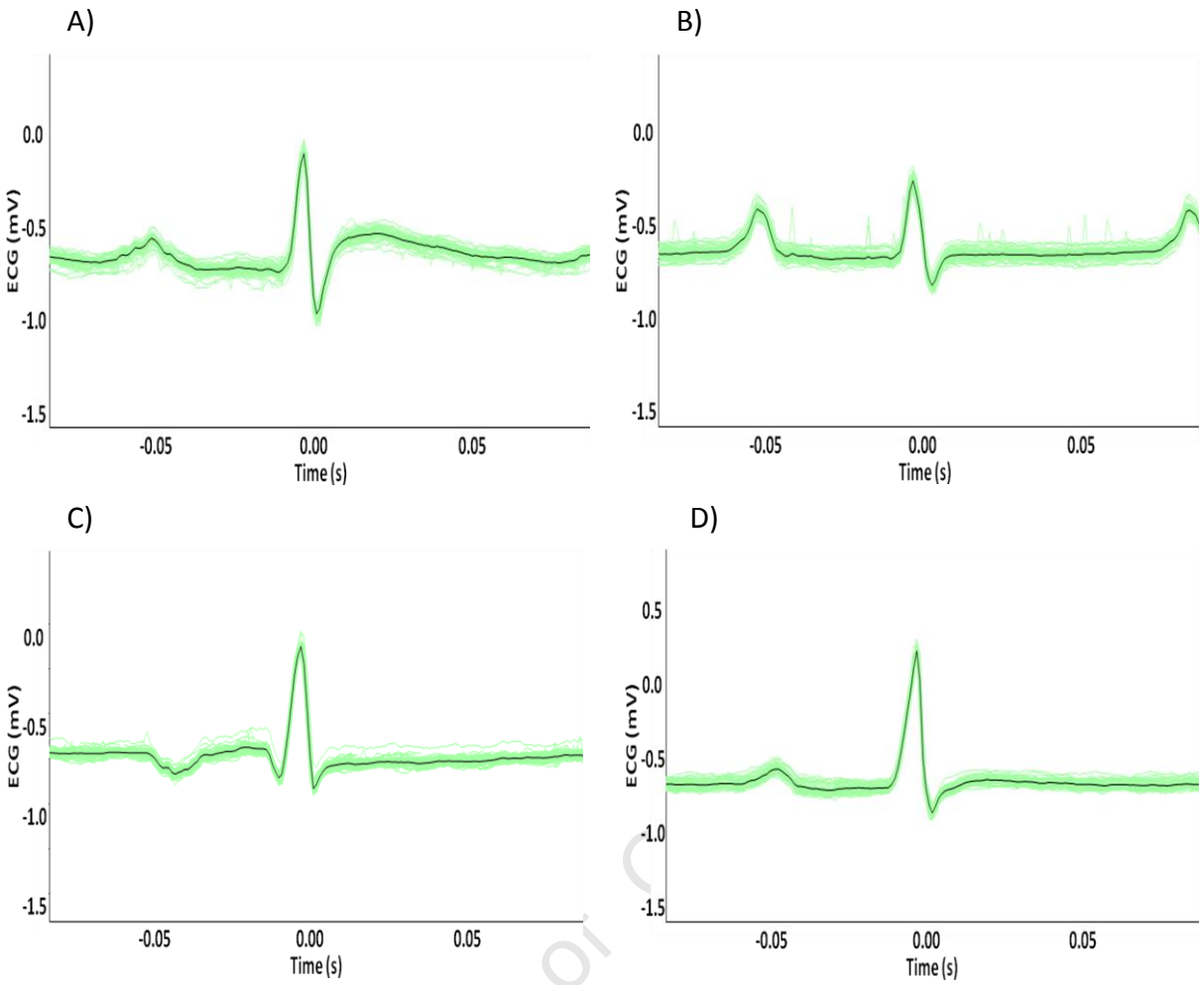


Figure 31: Effects of isoprenaline and ethanolamine on electrical activity. Superimposed original lead II ECG tracings from control (A), isoprenaline (B), isoprenaline + Ethanolamine (C) and Ethanolamine (D) rats. Dark black lines are the average tracing for n=8 rats, n=9 rats, n=10 rats and n=8 rats respectively. Green lines indicate traces from individual rats.

4.4.2.3 Isoprenaline and Ethanolamine do not Affect Haemodynamic Parameters

Figure 32 shows the effects of ISO and Etn on arterial systolic (A) and diastolic (B) BP. The results indicate that there was a trend for ISO to induce arterial hypotension compared to control rats (systolic BP control vs. ISO $P<0.054$; diastolic BP control vs. ISO $P<0.04$) and that the trend was abolished when the rat received both ISO + Etn.

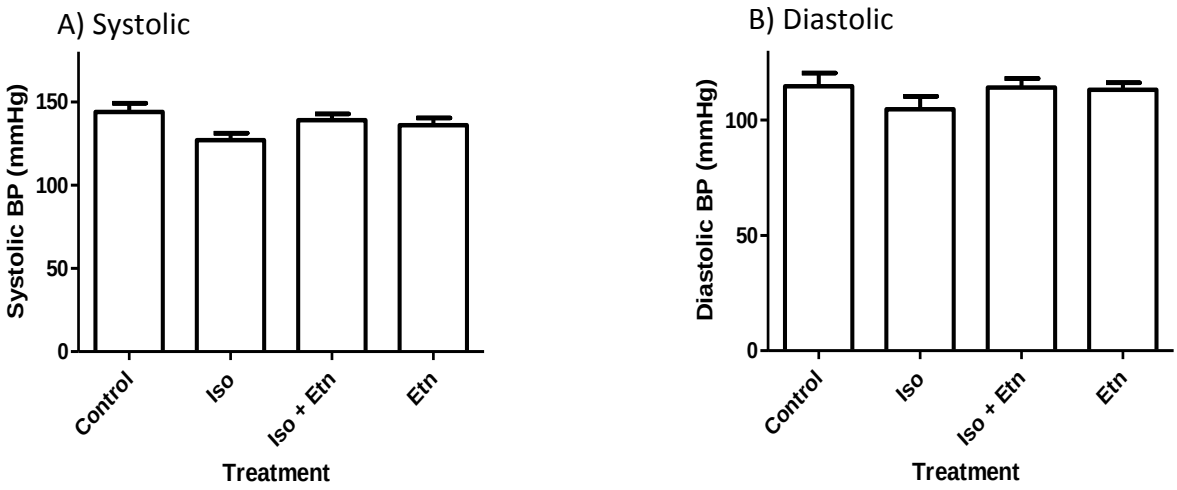


Figure 32: The effects of isoprenaline and ethanolamine on systolic (A) and diastolic (B) blood pressure parameters, measured on the 8th day of isoprenaline treatment.

4.4.2.4 The Effects of Isoprenaline and Ethanolamine on Cardiac and Non-Cardiac Structures

Administration of ISO did not cause a significant increase in the HW/BW ratio; however, the trend ($P<0.07$) to increase was clear (Table 11). Administration of both ISO + Etn caused a significant increase in the HW/BW ratio ($P<0.05$ vs. control) indicating hypertrophy of the myocardium. A trend was also observed for the lungs/BW ratio whereby ISO increased the ratio ($P<0.055$ vs. control) and ISO + Etn tended to decrease the lungs/BW ratio back towards control weights. Etn administered alone did not cause any significant difference to the organ weights or BW gained compared to control rats. Neither ISO nor Etn caused any significant gross structural alterations or weight changes in the liver, lungs, kidneys and adrenal glands.

Table 11: Alterations in body weight and organ weights in control, isoprenaline and ethanolamine treated rats.

Characteristic	Control	ISO	ISO + Etn	Etn
BW Gained (%)	11.94 ± 1.13	15.72 ± 2.43	12.23 ± 1.25	9.76 ± 1.48
HW/BW ratio	4.40 ± 0.00	5.24 ± 0.10	5.54 ± 0.20*	3.97 ± 0.20
Liver/BW ratio	40.85 ± 1.00	41.08 ± 1.60	39.16 ± 0.60	40.99 ± 0.60
Lungs/BW ratio	3.06 ± 0.10	3.46 ± 0.00	3.29 ± 0.20	3.20 ± 0.10
Kidneys/BW ratio	6.50 ± 0.20	5.84 ± 0.10	6.00 ± 0.10	6.86 ± 0.10
Adrenals/BW ratio	0.20 ± 0.00	0.19 ± 0.00	0.24 ± 0.00	0.19 ± 0.00

*P<0.05 (treatment vs. control)

4.4.2.5 Isoprenaline and Ethanolamine do not Alter Lipid Peroxidation Parameters

Lipid peroxidation was assessed in terms of CD and TBARS (Fig. 33A and B). There was no difference observed with the CD between ISO and control rats respectively (43.5 ±3.9 µmol/L vs. 45.9 ±1.2 µmol/L). Treatment with Etn alone or in a combination with ISO resulted in no significant difference to the CD. There was a trend for ISO to reduce the amount of TBARS compared to control rats respectively (8.2 ±1.5 µmol/L vs. 9.7 ±1.2 µmol/L; P<0.08).

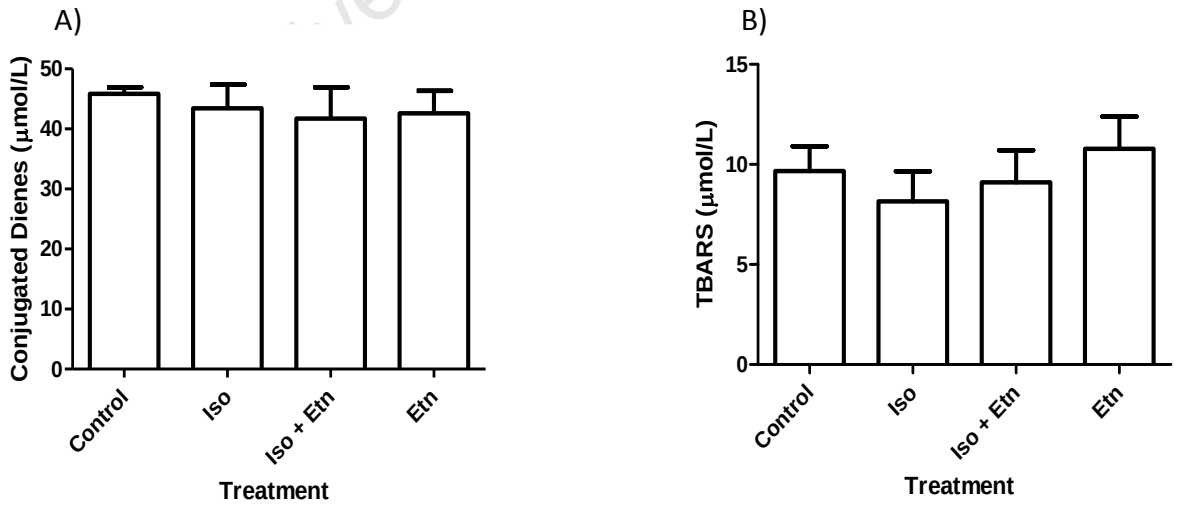


Figure 33: The effects of isoprenaline and ethanolamine on lipid peroxidation. CD (A) and TBARS (B) present in the rat plasma eight days after the initial isoprenaline treatment.

4.4.2.6 Ethanolamine Appears to Affect Depression Characteristics in Isoprenaline-Induced Cardiac Hypertrophy

Depression was monitored in rats using the FST. Administration of ISO appeared to affect depression in rats as shown by a decrease in the climbing time (Fig. 34A) and an increase in the floating time (immobility) (Fig. 34B) compared to control rats respectively (Control=73.8 ±19.1s, ISO=32.3 ±17.8s and Control=73.9 ±14.0s, ISO=112 ±19.7s). Pre-treatment with Etn appears to restore the climb time near to control values (Fig. 34A) and float time (Fig. 34B) back to control levels respectively (72 ±14.8s and 70.8 ±14.0s). There were no differences in the swimming variable. Treatment with Etn-alone created differences in depressive-like behaviours compared to control rats (decreased climb time, 33.9 ±12.2s and increased float time 125.1 ±19.8s).

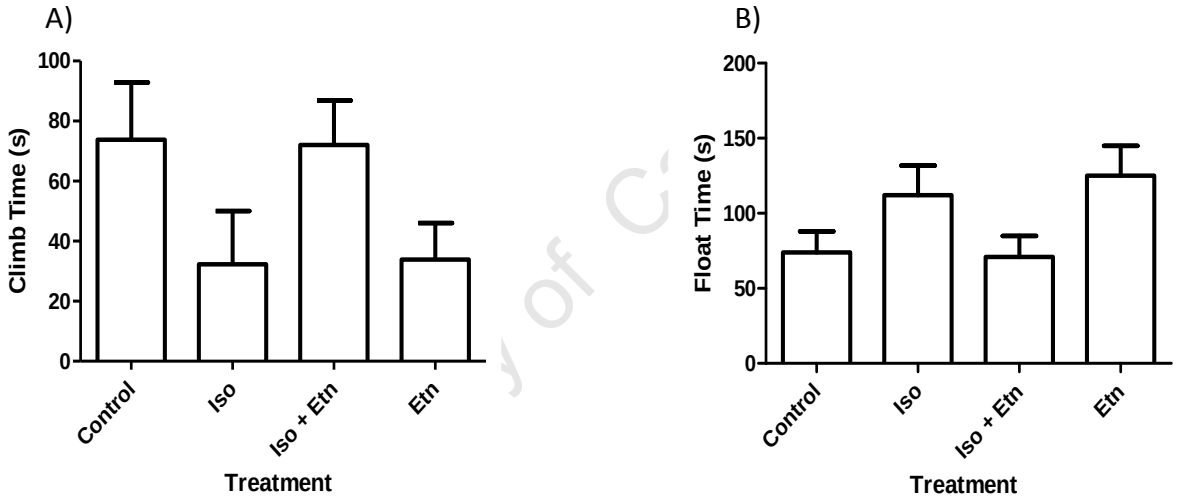


Figure 34: Depression assessment in rats as measured by the forced swim test (FST). Climb time (A) and float time (B) were the parameters analysed.

4.4.2.7 Pre-Treatment with Ethanolamine Appears to Affect Anxiety in Isoprenaline-Induced Cardiac Hypertrophy

Anxiety levels in the rat were measured using the EPM and the OF test. In the EPM, there was a difference between ISO and control rats in terms of a decrease in the distance moved (Fig. 35A) and an increase in the duration in the centre (Fig. 35B) compared to control rats respectively (Control=783.3 ±130.1 cm, ISO=664.4 ±133.5 cm and Control=75.2 ±14.7s, ISO=117.5 ±31.7s). Treatment with Etn elicited values different to ISO-treated rats in terms

of distance moved and duration in the centre respectively (787.7 ±67.0 cm and 73.9 ±34s). Figure 36A shows that ISO-treated rats spent less time in the open arms compared to control and ISO + Etn rats respectively (Control=58.8 ±14.1s, ISO=33.3 ±15.5s, ISO + Etn=46.5 ±7.1s). Figure 36B shows that ISO-treated rats spent less time in the closed arms, which is usually an indication of a decreased level of anxiety, compared to control rats respectively (119.5 ±22.2s vs. 158.3 ±24.7s). The results in all four parameters analysed suggest that treatment with Etn alone may have had an anxiety-like effect on the rats as shown by a slightly decreased distance moved and duration in the open arms, as well as a slightly increased duration in the closed arms compared to control rats respectively (697.7 ±118.7 cm, 37.2 ±11.6s and 188.6 ±36.6s).

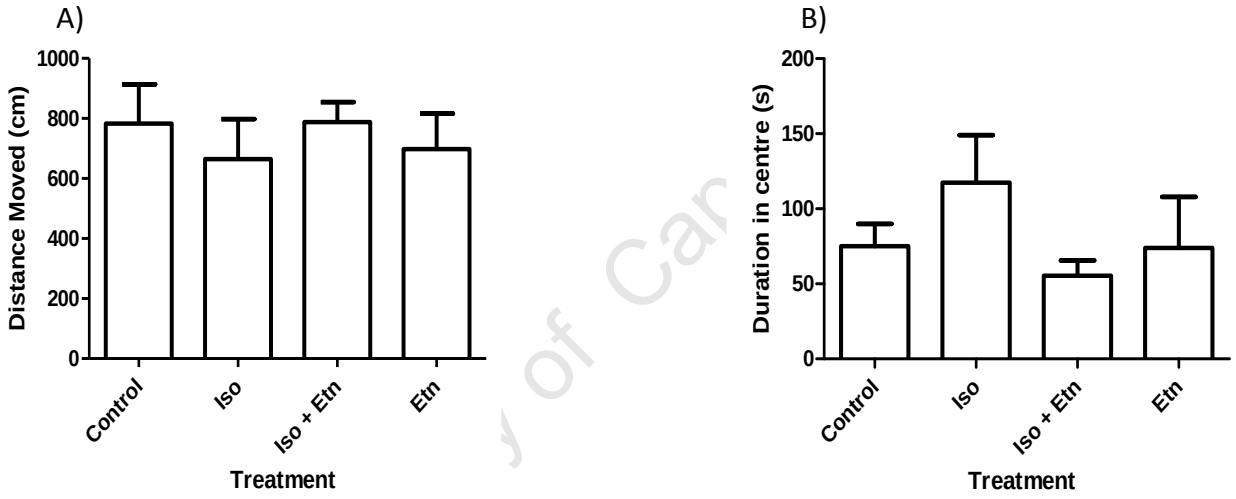


Figure 35: Anxiety in the rat as measured by the elevated plus maze, showing the distance moved (A) and the duration in the centre (B).

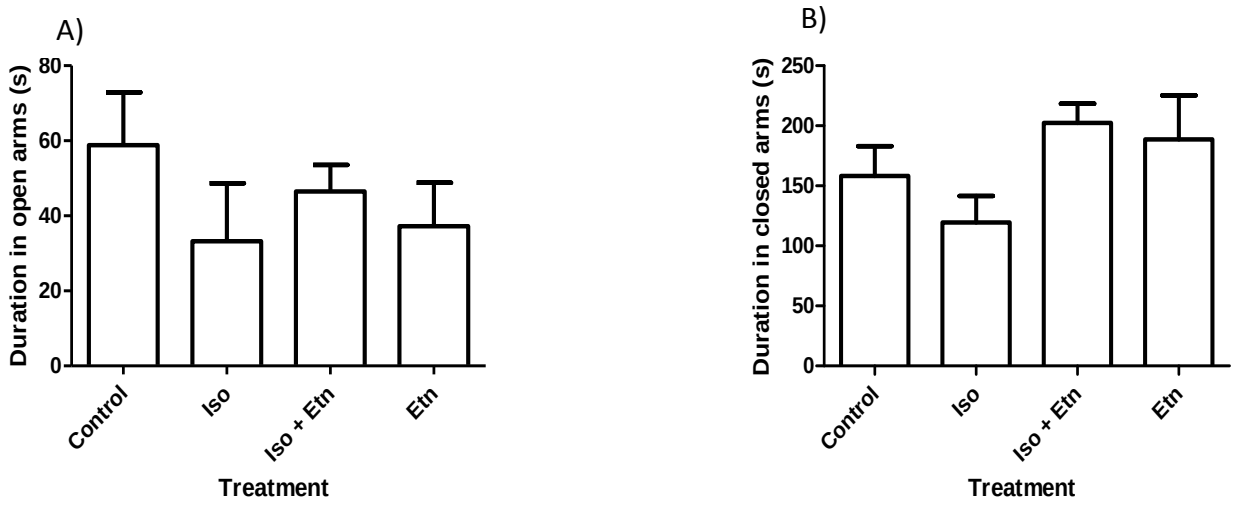


Figure 36: Anxiety in the rat as measured by the elevated plus maze, showing the duration in the open arms (A) and the duration in the closed arms (B).

In the OF test (Fig. 37A), there was a difference between ISO-treated rats and control rats in terms of distance moved (1934 ±147.3 cm vs. 1127 ±363.3 cm), indicative of an anxious state. Treatment with ISO + Etn created a difference in the distance moved parameter (1266 ±324.3 cm). ISO rats also moved slower (Fig. 37B) compared to control rats respectively (3.97 ±0.97 cm/s vs. 4.86 ±0.56 cm/s). Treatment with Etn had no effect on velocity compared to control rats (3.96 ±0.82 cm/s). Rats treated with Etn alone displayed a faster velocity compared to control rats (6.05 ±0.83 cm/s). There were no differences in the amount of time spent in the outer zone between groups (Fig. 37C), however, regarding the amount of time spent in the inner zone (Fig. 37D), there was a slight difference between the groups (Control=4.8 ±1.9s, ISO=1.8 ±1.0s, ISO + Etn=3.5 ±2.8s and Etn=6.4 ±3.0s). The high variation in this parameter in all groups makes extrapolation of the data difficult.

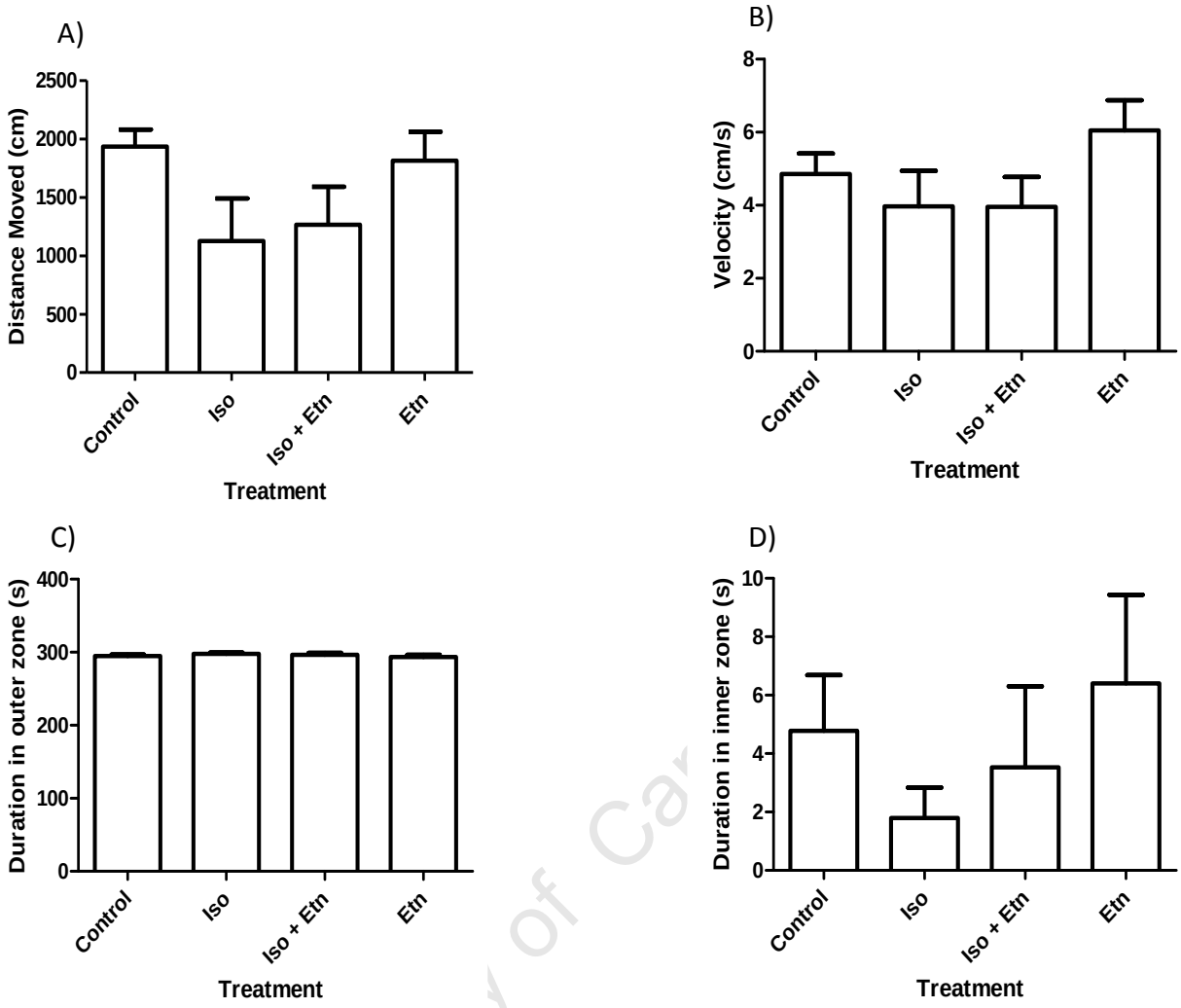


Figure 37: Anxiety in the open field as measured by measured by distance moved (A). velocity (B), duration in the outer zone (C) and duration in the inner zone (D).

DISCUSSION

ISO administered in an acute dose (for induction of MI) and a chronic dose (for induction of cardiac hypertrophy) caused necrosis and disruptions to the electrophysiology, haemodynamic and gross structural parameters of the heart. ISO also affected systemic lipid peroxidation, various organ weights as well as affecting BW gain or loss. ISO also altered the affective state of the rat. Pre-treatment with Etn lowered mortality in the MI model and increased the HW/BW ratio thereby affecting the electrical activity of the heart. The effects of Etn were also noted systemically, particularly concerning the lungs. Etn also increased the HW/BW ratio in the ISO-induced cardiac hypertrophy model and appeared to improve electrophysiological function and decrease heart rate. No difference was found in behaviour states of rats. As previous research has shown, a difference in depression and anxiety characteristics between rats treated with ISO and control rats was expected due to ISO-induced oxidative stress, BP alterations, apoptosis, augmentation of cytokines, electrolyte disturbances and mitochondrial dysfunction. Pre-treatment with Etn was expected to improve anxiety and depression characteristics through potential interactions with the renin-angiotensin-aldosterone system, apoptosis, TNF-α, voltage-activated K^+ channels, Ca^+ signalling and mitochondrial function as Etn has been shown previously to impact these systems. Mg^{2+} prevented ISO-induced hypotension and altered the electrical functioning of the heart. Mg^{2+} therapy also affected the kidney weights.

5.1 Characterisation of a Low Mortality Model of Isoprenaline-Induced Myocardial Infarction

In this study we characterised a rat MI model with decreased mortality compared to those in the literature. MI was induced by a single injection of a relatively low dose of ISO (67 mg/kg). The ISO model of MI is associated with a death rate due to the overdose of catecholamines. This protocol induced significant MI detectable by TTC staining, electrophysiological and haemodynamic changes, in a manner consistent with MI and also produced cardiac structural changes. There was also evidence of oxidative stress as indicated by the elevation of CD. The model produced low mortality and there were no deaths in our control groups unlike other methods of inducing MI such as coronary artery ligation (Johns and Olson, 1954; Guo et al., 2012). The cardiac changes and the low mortality

rate found in our study makes this a valuable, pharmacologically-induced, model of acute MI that provides results within 24 hours. Furthermore, our results validate and bring new understanding to the MI model previously used by Arteaga de Murphy et al. (2002).

The MI model described here produces a large infarction with a high survival rate. Other MI models in which ISO was used in doses of 85 mg/kg, 200 mg/kg or 300 mg/kg injected twice, 24 hours apart resulted in LDH leakage of 33%, 41% and 46% respectively (Sharma et al., 2001). In our study, a lower dose of ISO was used and induced a reproducible infarction as indicated by a TTC-negative staining of 64 ±3%. In addition, the mortality rate reported here is low (23%) compared to the rates reported in other studies where ISO was used to induce MI such as 50% (Crandall et al., 1981), 40-50% (Wexler 1979), 33% (Judd et al., 1969) and 31% (Mladenka et al., 2009). Singal et al. (1982) reported a similarly low mortality rate of 25% in an MI model in which a lower dose of ISO (40 mg/kg) was used, but in this model the dose needs to be repeated over two days.

The infarction caused by ISO was located primarily in the epicardium (Fig. 12A). It was expected that the cells demanding the greatest amount of energy (endocardial cells) would die first (Reimer and Jennings, 1979). However, Litovsky and Antzelevitch (1990) showed that the epicardium is more sensitive to ISO treatment than the endocardium. The authors postulate that the difference may be due to the prevalence of a transient outward current (I_{to}) in the epicardium as compared to the endocardium which they found in a previous study (Litovsky and Antzelevitch, 1988). It is important to note that the electrophysiological differences between the epicardium and the endocardium, caused by the domination of I_{to} in the epicardium, may affect the sensitivity of the two tissues to pharmacological drugs (Antzelevicth et al., 1999). Although the infarction was more visible in the epicardium, it must be remembered that unlike other methods of inducing infarction, such as coronary artery ligation which produces a clear ischemic region after staining, ISO will produce a global infarction and as such, diseased tissue will appear pale in colour compared to control rats.

ISO not only produced infarction but also induced a loss of BW and altered the gross structures of the heart and liver. It was found that ISO-treated rats showed a significant loss in BW 24 hours after treatment. Similar adverse effects and loss of BW with ISO has been reported previously (Wexler and Kittinger, 1963; Wexler, 1979) and was proposed to be due to the stress of myocardial necrosis or the catabolic state of the body because of altered protein metabolism (Wexler et al., 1971). The loss in BW after MI could be indicative of the development of early HF in rats (Faria et al., 2011). The increased heart weight could be attributed to the accumulation of myocardial edema, ground substance accumulation (Judd et al., 1969) or hypertrophy which compensates for the ISO-induced necrosis (Benjamin et al., 1989). Although the dry weight of the ventricles was not measured in our study to confirm the development of hypertrophy, there was no fluid accumulation present which would have increased the weight of the heart. The liver weight was also decreased in response to ISO administration. No further evaluation of liver function was conducted in our study, but there was no evidence of gross liver structural damage. Previous studies have shown that ISO has multiple effects on the liver: induction of necrosis (Grimm et al., 1998), increases in the activity of intracellular cyclic adenosine 3':5'-monophosphate (Pariza et al., 1977) and promotes lipid accumulation (Wexler, 1979). However, whether these detrimental effects change the liver/BW ratio is not clear.

The ISO-induced MI model also produces cardiac electrical alterations as evidenced by ECG changes in the Q-, R- and S-waves. The low-voltage ECG caused by ISO, represented by a decreased R-wave, is possibly due to loss of viable muscle tissue resulting from the treatment. A decreased S-wave is often observed in the hyperacute phase of MI (Ekmekci et al., 1961). The presence of a pathological Q–wave is indicative of an evolving MI (Thygesen et al., 2007), and suggests that the onset of infarction occurs early. An elevation of the ST segment would be expected in acute infarction as has been reported in other studies (Prabhu and Devi, 2006; Rajadurai and Prince, 2007). The ST segment in rats is difficult to assess because the beginning of the T-wave merges with the end of the QRS complex. Some authors even doubt the existence of an isoelectric ST segment in rats (Farraj et al., 2011). Therefore the ST segment was not measured in this study.

Along with electrophysiological changes, ISO also affected both left ventricular and arterial BP parameters. We showed that ISO significantly decreased the ventricular dP/dt min and dP/dt max, indicative of ventricular contractile and relaxation dysfunction. This is consistent with the findings of Jia et al. (2006) who showed that ISO decreased cardiac function as indicated by changes in dP/dt max. The duration of systole of the left ventricle was shortened in this study, possibly due to the non-contractile nature of the necrotic tissue caused by ISO. We also found that ISO lowered arterial systolic, diastolic and dicrotic notch BP. This is in accordance with previous literature by Chappel et al. (1959) who found ISO causes peripheral vasodilation which reduces blood flow to the worked myocardium. This decrease in blood flow causes temporary ischaemia followed by necrosis of the myofibers (Rona et al., 1959). This may be one of the mechanisms by which infarction, and other complications such as oxidative stress, is induced in the current model.

It is known that lipid peroxidation is detected in the deteriorating heart and administration of ISO causes myocardial oxidative stress (Wexler and Greenberg, 1978). The results of this study confirm that ISO causes oxidative stress, as measured by the elevated levels of CD in blood plasma 24 hours after administration of ISO. Sharma et al. (2001) confirmed the elevation of TBARS in the rat myocardium in response to ISO. TBARS were elevated because of the development of ischaemia and anti-oxidant retardation. In this study, we found a trend for TBARS values of diseased rats to drop below basal levels 24 hours after ISO administration. This is consistent with the findings of Roth et al. (1985) who assert that surplus malondialdehyde cannot be formed from the damaged myocardium.

5.2 Pre-Treatment with Ethanolamine may have Protected Against Isoprenaline-Induced Myocardial Infarction

Rats that were injected with ISO + Etn had lower mortality rates than rats that were injected with ISO alone. Previously Etn has shown to protect the isolated rat heart from ischaemia-reperfusion injury through activation of pro-survival pathways in the heart (Kelly et al., 2010). Protection of those rats was confirmed by a decrease in infarct size. The results of this study show that *in vivo*, Etn does not lower mortality by reducing infarct size in an ISO-induced MI model. Therefore the mechanism by which Etn lowered mortality *in vivo*

appears different to that which protected *ex vivo*. This required further investigation. The ECG waveform is modulated by ion channels but whether Etn affects ion channels in cardiomyocytes has not previously been investigated and therefore remains unclear.

ISO caused severe disruption to the electrical activity of the heart after 24 hours. ISO administration produced low voltage R-waves. The R-wave characterises ventricular depolarisation and abnormalities may represent myocardial edema and poor R-wave progression (PRWP) (DePace et al., 1983; Ramesh et al., 1998). PRWP is indicative of an anterior MI (DePace et al., 1983). Treatment with Etn did not restore R-wave values. ISO also induced pathologically large Q-waves which is an effect consistent with an evolving MI. The Q-wave will be present in the ECG when the infarcted muscle can no longer conduct the electrical current, causing a loss of force normally generated from the area. The result is an imbalance of force in the opposite direction from the inert region, rendering a large Q-wave (Patel et al., 2010). Pre-treatment with Etn restored the Q-wave value near to control levels, which may have possibly positively altered the evolution of the MI. ISO also affected the T_{peak}-T_{end} parameter which provides an indication of transmural dispersion of repolarisation (Antzelevitch et al., 1999). Treatment with Etn appeared to worsen this parameter, suggesting Etn may have altered the architecture of the myocardium. The amplitude of the T-wave was decreased when ISO was administered. The T-wave represents ventricular repolarisation; abnormalities may indicate disturbances in electrolyte balance, left ventricular hypertrophy or left bundle branch block (Pope et al., 2000). Pre-treatment with Etn improved the voltage of the T-wave, suggesting that Etn may interact with the balance of essential salts and the development of hypertrophy. Pre-treatment with Etn caused a shortening of the QTc compared to control rats, an alteration that was not viewed in ISO-only treated rats. The Bazett formula was used to calculate the QTc in the current study since the formula is widely used, but there are criticisms to this method in that it is more suitable for heart rates between 60-100 bpm and may overcorrect for fast heart rates (Malik, 2002; Surawicz and Knilans, 2008). In humans, while a prolongation of QTc is a risk factor for sudden cardiac death, a shorter than normal QTc is indicative of a short ventricular refractory period, predisposing the patient to ventricular fibrillation and arrhythmias (Gaita et al., 2003; Viskin et al., 2004; Straus et al., 2006). Etn treatment also

decreased the S-wave and P-wave amplitudes. These ECG disruptions suggest problems associated with atrial and ventricular depolarisation because of an electrolyte disturbance or injury to the cell membrane (Holland and Brooks, 1977; Mattu et al., 2000).

The ECG complications that occurred when ISO + Etn were co-administered that were not found when ISO was administered alone are intriguing. In the current study, Etn reduced mortality *in vivo* and was previously shown *ex vivo* to protect via STAT-3 activation (Kelly et al., 2010). The JAK/STAT pathway, which is integral in the development of cardiac hypertrophy (Kunisada et al., 1998), involves STAT-3 activation. It is therefore possible that administration of Etn *in vivo* may have interacted with STAT-3 to alter the architecture of the heart (inducing hypertrophy), reducing mortality and improving various ECG parameters but also resulting in several abnormal ECG recordings.

Administration of ISO increased the HW/BW ratio and pre-treatment with Etn augmented this increase. The increase in the HW/BW ratio over the 24 hour period could be attributed to myocardial edema, ground substance accumulation or hypertrophy as previously mentioned (Judd et al., 1969; Benjamin et al., 1989). Again, there were no indicators of fluid accumulation which would have increased the weight of the heart. Laine and Allen (1991) stated that for every 1% increase in myocardial water content, there will be a 10% decrease in cardiac function. Although cardiac function in terms of cardiac output and contractility were not measured, it is unlikely that the increased HW/BW can be attributed to myocardial edema as treatment with Etn decreased mortality and appeared to improve many ECG characteristics. Ma et al. (2005) states that a higher HW/BW ratio can suggest an increased level of compensatory (physiologic) hypertrophy. Therefore Etn may have caused a compensatory increase in the HW/BW ratio to allow the heart to keep up with the increased myocardial demand induced by ISO. With such alterations to the cardiac architecture, improvements in BP parameters were also expected.

After 24 hours from ISO administration, arterial hypotension had developed. This is in accordance with previous literature by Chappel et al. (1959) who found ISO causes peripheral vasodilation which reduces blood flow to the worked myocardium. This decrease

in blood flow causes temporary ischaemia followed by necrosis of the myofibers (Rona et al., 1959). In a review by McMullen and Jennings (2007) it is noted that in the rat, after infarction, the myocardium undergoes pathological remodelling and the rat develops eccentric hypertrophy. Thus the heart wall gets thinner and the ventricular cavity larger placing excess wall stress upon the myocardium. There is formation of new sarcomeres in an attempt to compensate for the increased wall stress and maintain the ejection fraction of the heart, however, eventually this hypertrophy becomes pathological and the function of the heart deteriorates (Zak, 1984). Treatment with Etn did not prevent the ISO-induced hypotension when assessed 24 hours after treatment. However, if Etn is causing an augmentation of a compensatory-type hypertrophy (i.e. production of excess sarcomeres); then improvements in BP may be viewed over a longer time point.

ISO causes cardiac-specific dysfunction, being a synthetic catecholamine that targets β-adrenergic receptors in the heart; however ISO also affects other organs (Rona et al., 1959; Kahn et al., 1969). Therefore the mechanisms by which Etn lowered mortality may not be limited directly to cardio-specific parameters. The effects of ISO on lung/BW ratio are poorly researched however; Kahn et al. (1969) states that ISO causes lung congestion. In models such as coronary artery ligation, there is an increase in the lungs/BW ratio due to the pulmonary edema associated with left ventricular dysfunction (Pasternak et al., 1992; Young et al., 1998; See et al., 2004). In this study we found that ISO caused a decrease in the lungs/BW ratio. ISO can increase Na^+ active transport which may cause fluid clearance in the lungs (Berthiaume et al., 1987; Jayr et al., 1994). This may explain the decrease in the lungs/BW ratio after 24 hours. Pre-treatment with Etn prevented the ISO-induced decrease in lungs/BW ratio which may suggest an interaction of Etn with Na^+ active transport. Rats injected with ISO also displayed a loss in BW. The reasons for this are uncertain however administration of an acute dose of ISO may cause anorexia due to a loss of appetite and metabolism alterations (Lora-Vilchis et al., 1988; Yamashita et al., 1994). Similar results have been observed previously and authors attribute the loss in BW to the catabolic state of the body due to altered protein metabolism and the stress of myocardial necrosis (Wexler et al., 1971; Wexler, 1979). The loss in BW after MI has been deemed indicative of the development of early HF in rats (Faria et al., 2011). However, Faria et al. (2011) measured

the BW of their rats seven days post-MI and as the rats in this study were weighed 24 hours after the cardiac insult, it seems unlikely that the development of HF would occur so rapidly. When rats were pre-treated with Etn, there was a further loss in BW, however to assess whether Etn may be impacting more heavily on the metabolism or eating behaviours of the rat than ISO, further studies monitoring caloric intake are required.

The loss in BW may also be associated with the increased oxidative stress caused by ISO that is brought about by an imbalance in the equilibrium of anti-oxidants (scavenger enzymes such as catalase and superoxide dismutase as well as non-enzymatic scavengers such as α-tocopherol and ascorbic acid) and free radicals. The results of this study indicate that ISO did not significantly elevate the levels of CD or TBARS in the rat plasma. However, a power analysis was conducted and it showed that the experiment had 70% power to detect differences between means with a significance level of 0.05. Therefore if the numbers of rats per group was increased, the results may have reached significance (which would be consistent with the lipid results in the characterisation of the model study Fig. 15), as it was expected that ISO would increase markers of lipid peroxidation in the plasma. The discrepancy between this study and previous studies reporting that ISO causes an increase in oxidative stress markers (Karthikeyan et al., 2007; Mukherjee et al., 2010; Patel et al., 2010), can also be explained by the different time point for measurement of the markers and the differing methods. The study conducted by Karthikeyan et al. (2007) analysed TBARS after 48 hours of ISO treatment and noted a significant difference. The method also differed in that ISO was administered for two consecutive days. Mukherjee et al. (2010) and Patel et al. (2010) both analysed homogenised myocardium, not circulating plasma, and found significant differences in lipid peroxidation markers. It was also shown by Roth et al. (1985) that levels of TBARS were elevated after three hours of cardiac injury and by 24 hours had returned to basal levels due to surplus malondialdehyde not being produced from damaged myocardium. Therefore the time point of lipid peroxidation measurements may explain the unexpected results obtained in this study.

5.3 The Effects of Magnesium Pre-Treatment on Isoprenaline-Induced Myocardial Infarction

Mg^{2+} has long been used in management of cardiac dysfunction and is assumed to protect via antitachydysrhythmic properties given the crucial role Mg^{2+} plays in regulating ion channels in cardiac cells (Rasmussen et al., 1986; Ceremuzyuski et al., 1989; Gwanyanya et al., 2004). However, the influence of Mg^{2+} on arrhythmias in MI has been disputed (Roffe et al., 1994). In humans, Mg^{2+} is also suggested to be cardioprotective by inhibiting platelet aggregation (Shechter et al., 1995), inducing arterial vasodilation to reduce afterload on the heart (Yusuf et al., 1993), increasing the energy production in the myocardium by improving mitochondrial ATP synthesis (Hearse et al., 1977; Rasmussen et al., 1988), as an anti-oxidant (Garcia et al., 1998) and by decreasing the catecholamine-induced Mg^{2+}-Ca^{2+} shifts (Rasmussen et al., 1988). This study investigated the potential cardioprotective effects of increasing the circulating Mg^{2+} content pre-MI.

Due to the vast use of Mg^{2+} in cardiovascular medicine, it was expected in this study that Mg^{2+} is a potent Ca^{2+} blocker (Altura and Altura, 1987) and is able to conserve energy in the myocardium by improving mitochondrial function (Hearse et al., 1977; Rasmussen et al., 1988). It was expected that Mg^{2+} would decrease infarct size. In dogs, Mg^{2+} reduces the infarct size in a model of coronary artery ligation (Barros et al., 1995) but the authors concluded that the decreased necrosis in Mg^{2+} treated dogs was attributed to haemodynamic and metabolic alterations. In the current study Mg^{2+} therapy did affect the haemodynamic parameters of the left ventricles.

Administration of ISO caused a decrease in the maximum pressure of the left ventricle, dP/dt min and dP/dt avg. Pre-treatment with Mg^{2+} restored these parameters near to control levels. Barros and Pileggi (1991) assert that Mg^{2+} will antagonize the effects of β-adrenergic stimulation, such as that with ISO administration, and therefore will combat the ISO-induced hypotension. As dP/dt min is a measure of left ventricular diastolic function, the results suggest that Mg^{2+} improved myocardial lusitropy. A possible mechanism for this involves suppression of the ISO-induced Ca^{2+} overload in the myocardium by Mg^{2+} (Jin et al., 2007). The influx of extracellular Ca^{2+} through voltage-dependant Ca^{2+} channels can be inhibited by the presence of excess Mg^{2+} (Yamaoka and Seyama, 1996). Mg^{2+} also modulates

Ca^{2+} release from the sarcoplasmic reticulum (Meissner and Henderson, 1987). As haemodynamic disturbance is often caused by Ca^{2+} overload in the myocardium (Allard et al., 1994), it can be speculated that the improved haemodynamic function associated with ISO + Mg treated rats can be attributed to the Ca^{2+} blocking activities of Mg^{2+}.

Rats treated with ISO + Mg also experienced an increased systolic duration. If the prolongation of the systolic duration is at the expense of the diastolic duration, the patient is at risk for CAD and HF due to a shortened relaxation phase in the heart (Plehn et al., 2008; Smedsrud et al., 2012). However, in this study, rats treated with both ISO + Mg did not display any significant differences in the diastolic duration and therefore the prolonged systolic duration may not be detrimental. It was anticipated that ISO would alter the LVEDP, an indication of ventricular filling abnormalities, as is widely shown by previous studies inducing MI in rats (Grimm et al., 1998; Teerlink et al., 1994; Mohanty et al., 2009). The results of this study do not show any differences in LVEDP between ISO and control rats. A possible explanation for this is that alterations in the LVEDP is generally a pre-requisite for cardiac failure (Mielniczuk et al., 2007) therefore possibly over a time point longer than 24 hours, there may have been alterations in this parameter in the ISO-treated group. There were also no disruptions to the contractility index in ISO-treated rats, which may explain the unexpected LVEDP results as the two variables are intimately linked. The greater the decrease in contractility, the greater the end diastolic pressure-volume relation is disturbed (Takano and Glantz, 1995). We expected ISO to decrease contractility of the left ventricle as has been displayed in previous literature (Ojha et al., 2010). The study conducted by Ojha et al. (2010) however, used a model of MI that requires two injections over a 48 hour period. So again, perhaps the time point for the current study should have been extended to observe if the single injection model produces lowered contractility.

Along with treatment of hypertension, Mg^{2+} therapy is perhaps most prevalent when treating arrhythmias and other electrophysiological disorders. In this study, ISO caused a decrease in the T_{peak}-T_{end} interval therefore indicating disruption to the transmural dispersion of ventricular repolarisation and possible development of Torsades de Pointes (Antzelevitch et al., 1999; Can et al., 2007). Some authors argue however that T_{peak}-T_{end}

should not be used as a direct measure of transmural dispersion of repolarisation but rather used as an index of dispersion that can be used to predict fatal arrhythmias (Milberg et al., 2005; Xia et al., 2005). Treatment with Mg^{2+}, in this study, did not prevent T_{peak}-T_{end} disruption even though Mg^{2+} therapy is widely used as a therapy for Torsades de Pointes and arrhythmias (Abraham et al., 1987; Tzivoni et al., 1988). The quantity of arrhythmias present in rat ECGs was not directly analysed in this study and could form the basis of future work, including the monitoring of ECG parameters in the conscious rat. Here, the recordings were taken over a 20 min period and therefore only the parameters which predict the occurrence of arrhythmias was assessed.

ISO also caused pathologically large Q-waves, indicative of an evolving MI and treatment with Mg^{2+} lessened the Q-wave amplitude. In humans, low serum levels of Mg^{2+} correlate with increased occurrence of Q-wave infarction and mortality (Booth et al., 2003). Therefore it was expected that treatment with Mg^{2+} before MI should restore Q-waves near to normal levels. ISO caused a decrease in the R-wave which is indicative of the presence of necrotic, non-contractile tissue. Treatment with Mg^{2+} did not rectify the low-voltage R-wave, therefore not preventing necrosis of the myocardium which was confirmed with TTC staining. Rats treated with both ISO + Mg displayed further disruptions to the T-wave and S-waves compared to ISO-only treated rats. These disturbances may suggest that Mg^{2+} therapy did not restore any electrolyte imbalance incurred by ISO. Interestingly, the condition T-wave alternans is associated with hypomagnesemia and is modulated by Ca^{2+} and K^{+} transport across the myocardial membrane (Kleinfeld and Gross, 1956; Rickets et al., 1969). Therefore it was expected that raising the serum levels of Mg^{2+} pre-MI may reduce T-wave complications.

ECG disruptions caused by ISO were accompanied by a loss in BW. Treatment with Mg^{2+} did not prevent the BW loss. As Mg^{2+} is involved in over 300 enzymatic processes impacting on metabolism and protein synthesis (Vernon, 1988; Elin, 1994), it was expected that pre-treatment with Mg^{2+} would prevent the ISO-induced loss in BW. As the results were unexpected, this may provide some clarity as to the reasons for BW loss in this model of

ISO-induced MI, not being of a metabolic disturbance but rather of an alteration in eating habits. The effect of Mg^{2+} on the appetite of rats is not well researched.

Mg^{2+} therapy also had no impact on the ISO-induced increase of the HW/BW ratio. The increased HW/BW ratio seen in ISO treated rats could be attributed to hypertrophy of the myocardium (Teerlink et al., 1994). Mg^{2+} regulates many cation channels in the body including transient receptor potential (TRP) channels in the heart which may be involved in the development of hypertrophy (Guinamard et al., 2007). It is known that the channel, TRPM7, which is widely expressed in cardiac tissue, is regulated by Mg^{2+} (Mubagwa et al., 2007). It was therefore expected that Mg^{2+} should impact on the HW/BW ratio however, the time point of this effect may be after 24 hours. Treatment with ISO + Mg caused a significant decrease in the kidney/BW ratio. The kidney/BW ratio can be used as an index of normal growth (Teerlink et al., 1994), however whether this ratio depicts growth over a 24 hour period is inconclusive and therefore no speculations can be made as to the effects of Mg^{2+} therapy on growth rate. The kidneys are essential in maintaining Mg^{2+} homeostasis in the body and any excess Mg^{2+} will be filtered through the kidneys. Therefore possibly by increasing the amount of circulating Mg^{2+} two hours prior to the induction of MI, the kidneys had already begun filtering the excess Mg^{2+}. Designing the study so that MI was induced closer to Mg^{2+} administration, or that MI was induced later than two hours after Mg^{2+} therapy, may have resulted in a positive effect on the kidney/BW ratio.

The time point of the experiment of 24 hours could also have affected the level of lipid peroxidation in rats treated with ISO which was not significantly different to control rats. The discrepancy between the results of the ISO-treated rats in this study compared to other studies has been discussed above and includes different method of assessing lipid peroxidation as well as different time points. Should the ISO-model however have increased the amount of CD and TBARS in the plasma it would be expected that Mg^{2+} would lower these values. Lipid peroxidation will impair the myocardial cell membrane resulting in electrophysiological and haemodynamic dysfunction. Treatment with Mg^{2+} protects against several ISO-induced haemodynamic and electrophysiological dysfunctions, but possibly not

through its known anti-oxidant mechanisms as has previously been suggested (Garcia et al., 1998; Jin et al., 2007).

5.4 The Effects of Ethanolamine Pre-Treatment on Isoprenaline-Induced Cardiac Hypertrophy

Histopathological examination of cardiac tissue revealed that administration of ISO caused severe necrosis of the myocardium, separation of cardiomyocytes, damage to myocardial cell membranes and an infiltration of inflammatory cells. The hearts of control rats showed normal myocardial membrane integrity with no infiltration of inflammatory cells. Similar results have been reported from other studies (Kumar et al., 2009; Patel et al., 2010).

The necrosis caused by ISO also affected the electrical capacity of the myocardium. Treatment with Etn appeared to positively affect the electrophysiological function of the myocardium, namely by shortening the ISO-induced prolongation of the QRS interval and restoring the amplitude of the R-wave. ISO caused a delayed QRS interval which may indicate that a bundle branch block was present (Akhtar et al., 1988). An injured fascicle, in this study due to a catecholamine overdose, would not conduct electrical impulses accurately. The impulse would be slowed down and the direction imprecise, resulting in dyssynchrony and prolongation of depolarisation ultimately reducing the ejection fraction. Holland and Brooks (1977) state that complications with the QRS complex are a result of an injured cell membrane. Pre-treatment with Etn rectified the block and as such Etn, which is found endogenously in the cell membrane lipids, may be enhancing the integrity of the cell membrane under stress-induced, pathological conditions. ISO caused a depression of the R-wave which may represent myocardial edema and an anterior MI. Treatment with Etn appeared to restore this back to control levels. When exogenous Etn is ingested, it alters the composition of phosphatidylethanolamine, a lipid present in plasma membranes (Kano-Sueoka and Errick, 1981; Murakami et al., 1982). Therefore electrophysiological protection from a disturbed QRS complex and a decreased R-wave may be brought about by Etn's actions on the myocardial cell membrane.

Treatment with Etn also caused a decrease in the resting heart rate of the anaesthetised rat. A decrease in heart rate has been associated with a baroreceptor reflex activation causing a negative-feedback loop with high BP lowering the heart rate (Coleman, 1980). Further studies are required which assess the BP intermittently and correlates it to heart rate in order to evaluate the impact of ISO and Etn on the baroreceptor reflex. A possible explanation for the decreased heart rate would be an excessive stimulation of the parasympathetic nervous system; however, it is known that a barbiturate, like sodium pentobarbitone, inhibits parasympathetic reflex vasodilation (Ito et al., 1998). As the heart rate was assessed in the anaesthetised rat, the influence of the anaesthetic cannot be forgotten and therefore the alterations in heart rate cannot be attributed to parasympathetic stimulation in this study. Heart rate variability, and consequently the degree of parasympathetic stimulation, is difficult to measure in anaesthetised and ventilated rats due to the numbing effects of barbiturates and the influence of controlled breathing on the parasympathetic nervous system. To investigate whether the parasympathetic nervous system was compromised, the heart rate variability would need to be measured in a conscious rat.

Rats treated with both ISO + Etn also had significantly higher HW/BW ratio's than diseased and control rats. The model used in this study to induce hypertrophy is well-established and is widely cited. Previous studies have confirmed hypertrophy using this model as early as 5 days of treatment (Rossi et al., 1981) and some studies use a smaller dose of ISO over the 7 day period and can confirm hypertrophy (Tang et al., 2011). A higher HW/BW ratio in a chronic ISO model usually indicates pathological hypertrophy due to an increased left ventricular mass/BW ratio (Judd et al., 1969; Benjamin et al., 1989) and this can be confirmed by histological (Fig 29) and electrophysiological analysis (Table 11). It was noted by Ma et al. (2005) that a higher HW/BW ratio may suggest physiologic hypertrophy. If in fact the rat hearts in this study treated with ISO + Etn experienced physiologic hypertrophy rather than pathological hypertrophy then it is possible that the increased HW/BW ratio may represent a more efficient heart and as such would be able to beat slower, producing the same amount of force. Staining with H&E however did not show differences in the amount of necrosis to the myocardium between ISO and ISO + Etn treated rats. Alterations

to the architechture of the heart may not always be reflected by changes in BP as ISO can also affect contractility masking any HW/BW ratio change-induced pressure alterations. As such, confirmation of the cardiac output of the heart is required using echocardiography and/or pressure-volume loops.

Along with cardiac-specific effects, ISO also affects the rat systemically. It was anticipated that ISO administered chronically would increase the BW gain as shown previously by Kudej et al. (1997). This weight gain can be attributed to an increased consumption of food resulting in increased brown fat and muscle mass due to the β-adrenergic stimulation (Geleon et al., 1988; Perez-Llamas and Zamora, 1991). Previously Etn has been shown to improve hypercholesterolemia in rats and impact on cholesterol in the serum (Kume et al., 2006). It was therefore expected that treatment with Etn would modulate the excess weight gained in ISO treated rats. Food consumption was not measured in this study and is a necessary measurement to postulate any effects of Etn on weight gain. Unlike acute ISO administration, there was a trend for chronic administration of ISO to cause an increase in the lungs/BW ratio. This is consistent with other models of cardiac dysfunction whereby left ventricular dysfunction causes pulmonary edema (Pasternak et al., 1992; Young et al., 1998; See et al., 2004).

Another systemic measure obtained in this study was lipid peroxidation levels in the plasma. Geng et al. (2004) noted that ISO injected chronically in rats causes increased CD in the rat plasma. In this study of hypertrophy, there were no differences in the CD produced. A possible explanation for this is that the method of inducing cardiac hypertrophy adopted by Geng et al. (2004) included varying doses of ISO, starting at 20 mg/kg on the first day and decreasing to 3 mg/kg on the last day. As the rats in this study received a constant 5 mg/kg injection for seven consecutive days, it may be argued that the dose was too low initially to cause marked increase in CD. This study also did not render any differences in TBARS after seven days of ISO administration. Studies such as that by Jaiswal et al. (2010) yielded significant increases in TBARS; however ISO was administered for 14 days in accordance with the method described by Arthur and Belcastro (1997). Therefore the model used in this

study may not have induced a severe enough form of cardiac hypertrophy to elicit significant alterations in lipid peroxidation markers.

The link between CVD and neurological dysfunction is an emerging field of research which highlights the intimate link between the heart and the brain. Much focus has been given to the effects of depression and anxiety on heart disease and usually the disruptions occur through cytokine or ROS-dependant pathways (Goshen et al., 2008; Dean et al., 2010; Sanders and Maze, 2010; Rousseau et al., 2012). This study focused on the development of depression and/or anxiety after the cardiac insult caused by ISO. Previous research on the link between cardiac insult and behavioural deficits suggests that CVD can alter affective state (Prickaerts et al., 1996; Grippo et al., 2003; Wann et al., 2007; Rousseau et al., 2012). We therefore expected that rats treated with ISO would display anxiety-like behaviours that were significantly different to control rats.

Overall, no significance was obtained for any of the behaviour parameters tested. While the sample size may be large enough to assess for differences in cardiac and systemic parameters, it may be too small to assess behaviour differences which have a higher variation between each rat. For example, a power analysis was conducted on the climb time parameter measured in the FST that revealed that the experiment had a 20% power to detect a difference between means of control and ISO-treated rats with a significance level of 0.05. It is essential in future studies to ensure the sample size is adequate when testing behavioural characteristics. Another possible explanation for the lack of significance found in behavioural measures is that there is contention over the FST as a measure of depression. Many scientists argue that immobility in the FST may represent learning in the rat. For example: the rat has learnt it is unable to escape from the cylinder and rather conserves energy awaiting the return of the experimenter (Petit-Demouliere et al., 2005). In this case, another test for depression such as the sucrose-preference or social interaction tests should have been conducted in addition to the FST.

Post-MI, ISO increases oxidative stress (Wexler and Greenberg, 1978), which is known to induce anxiety-like behaviour in mice (Hovatta et al., 2005; Rammal et al., 2008). In 2005,

Hovatta et al. discovered that anxiety is regulated by glyoxalase 1 and glutathione reductase 1, two potent anti-oxidant enzymes. Measurement of anti-oxidant enzymes in this study was not conducted however in a study by Tanwar et al. (2010) it was noted that ISO did not affect levels of glutathione reductase 1. More research is required to assess whether ISO-induced anxiety affects anti-oxidant enzyme status. In a study by Bouayed et al. (2007), increased levels of oxidative stress in the peripheral blood granulocytes was correlated to increased anxiety in the rat. As the current study found no differences in lipid peroxidation markers in the plasma, the mechanisms by which ISO caused differences to anxiety in the rat require further investigation, possibly by way of assessing the lipid peroxidation status of different organ tissues. Rammal et al. (2008) measured oxidative stress markers in both the brain and the blood. Higher oxidative stress levels were found in the neuronal and glial cells of the hippocampus and cerebellum as compared to peripheral blood cells. The effect of Etn on oxidative stress is ambiguous because the model failed to induce adequate alterations to oxidative stress levels. Recently, dietary anti-oxidants have been deemed to exert both cytoprotective and anxiolytic actions (Bouayed et al., 2007; Vignes et al. 2006). Testing Etn's effects in a model which accurately induces oxidative stress and subsequent anxiety may shed more light on this issue.

Anxiety has also been linked to alterations to BP parameters. ISO affects both arterial and left ventricular BP parameters when administered acutely (Fig. 14, Table 5) but the effect of ISO on arterial BP was not clear in this model. Whether ISO affected left ventricular BP should have been investigated due to the cardiac remodelling of the ventricles that is associated with the model. Perhaps alterations in left ventricular BP would have elicited more apparent results than arterial BP measurements. It was expected that ISO would decrease arterial BP parameters. In a model of MI, coronary artery ligation, Prickaerts et al. (1996) found that anxiety was increased in rats post-MI. The anxiety was reduced when rats were treated with Captopril, a powerful angiotensin-converting enzyme inhibitor used in the modulation of hypertension. Angiotensin-converting enzyme interacts with the renin-angiotensin-aldosterone system to modulate extracellular volume and cause vasoconstriction. Interestingly, the renin-angiotensin-aldosterone system is involved in early remodelling of the myocardium after MI (Hall, 1996). Grimm et al. (1998) showed that ISO

affects the renin-angiotensin-aldosterone system which impacted on the progression of cardiac remodelling to HF. Therefore in the current study, ISO may be affecting the renin-angiotensin-aldosterone system. Saavedra et al. (2005) found that components of the renin-angiotensin-aldosterone system are implicated in anxiety disorders in rats. As it was expected in the current study that ISO would increase anxiety levels, the effects of ISO on the renin-angiotensin-aldosterone system should have been investigated. Pfeiffer et al. (1971) identified Etn as a powerful renin inhibitor that mediates renin-angiotensin-aldosterone system functioning. It is uncertain whether Etn interacts with the renin-angiotensin-aldosterone system in the current study, as alterations in BP parameters were not clear in the model of ISO-induced cardiac hypertrophy (Fig. 32).

Wann et al. (2007) discovered that it was due to apoptosis in the limbic system that rats developed depression after MI. Administration of ISO causes apoptosis in the rat myocardium which occurs from 12 hours to seven days after administration (Shizukuda et al., 1998). In the same paper, Shizukuda et al. (1998) also state that with an increase of cardiomyocytes in hypertrophic conditions, there is a subsequent increase in apoptotic cells. In a paper by Matas et al. (2007), anandamide was shown to protect against low serum induced apoptosis in neuroblastomas. This protection occurred because anandamide was degraded to Etn. The pathway from anandamide to Etn appears to play an important role in protection against apoptosis as Surette et al. (1996) administered exogenous Etn (100 µM) to cells and found no effect on reversal of apoptosis. As apoptosis was not measured in the current study, only speculations can be made that Etn protects from apoptosis *in vivo*.

The pathways by which cardiac pathology may induce depression and anxiety in rats is relatively unknown, yet in a review by Pasic et al. (2003), cytokines were highlighted as playing a major role in the development of depression. Cytokines are released during reperfusion and have receptors in various brain areas such as the hypothalamus, hippocampus and the amygdala (Vitkovic et al., 2001; Francis et al., 2004). Increased catecholamine activity can also cause the increased expression of cytokines (Burger et al., 2001; Pasic et al., 2003; Rousseau et al., 2012). Therefore administration of ISO causing high levels of circulating catecholamines may induce depression via the cytokine pathway.

Rousseau et al. (2012) summarises that cytokine augmentation can limit neurogenesis, alter neurotransmitter metabolism and induce apoptosis. The interaction of Etn with cytokines was not explored in this study, but it was expected that Etn would affect cytokine release. A review by Lecour and James (2011) summarises that cytokines, particularly TNF-α, cause the activation of the SAFE pathway and exacerbate the failing heart condition. STAT-3 is actively involved in the SAFE pathway and as Etn protects via STAT-3 activation, it would be expected in this study that Etn would influence the expression of cytokines and subsequently affect the behaviour of the rat.

Electrolyte disturbances are involved in the development of affective disorders such as anxiety and depression (Baer et al., 1970; Barraclough, 1997). ISO directly impacts on myocardial electrolytes (Kahn et al., 1969) and as such it was presumed in this study that ISO would cause significant alterations to the depressive and anxiety states of the rats. In neuronal cells, Etn is known to alter the sensory excitability by modulating voltage-activated K^+ channels and interacting with intracellular Ca^+ signalling (Khairy et al., 2010). The interaction of Etn with essential salts was not directly measured in this study; however, Etn did have an effect on ECG data as mentioned above. As the ECG waveform is largely governed by electrolyte (Na^+, K^+, Ca^+ and Cl^-) movements across the cell membrane, it is expected that Etn could directly interact with these circulating essential salts. The urine of rats should have been harvested and the quantity of Na^+, K^+, Ca^+ and Cl^- should have been measured in order to provide a more accurate representation of ISO and Etn's actions with these electrolytes.

The pathology of affective illnesses also includes dysfunction of mitochondria (Kato and Kato, 2000). Administration of ISO causes mitochondrial swelling and dysfunction due to the uptake of Ca^{2+} (Ferrans et al., 1964; Bloom and Cancilla, 1969). It was therefore presumed in this study that ISO would cause marked alterations to the affective state of the rat. There is an unclear link between Etn and mitochondrial function, which can regulate phospholipid metabolism (Modica-Napolitano and Renshaw, 2004). The effects of ISO and Etn on mitochondrial function were not explored in this study but such investigations may have

been useful in determining Etn's effects on neurological dysfunction associated with heart disease.

Etn administered alone appeared to negatively affect anxiety and depression characteristics of the rat. It was noted that the rats treated with Etn alone were often partnered with a diseased rat (either ISO-only or ISO + Etn) that had died due to ISO treatment, whereas control (saline-treated) rats were often partnered with a diseased cage mate that survived. This was unintentional as all rats were presumed equally healthy and equally sensitive to ISO treatment before any treatment began. Although not heavily researched, it is possible that the stress of a cage mate dying may impact on the anxiety and depression levels of the rat. This may also provide an explanation for the elevated TBARS in the plasma as anxiety has been linked to increases in oxidative stress (Liu et al., 1996; Eren et al., 2007; Kamper et al., 2009).

5.5 Limitations

a) The current study did not consider the quantity of food ingested by the rats. Analysis of food intake made have shed light onto the effects of ISO on eating behaviours, but also whether Etn and Mg^{2+} affected appetite or food metabolism.

b) In order to confirm hypertrophy, the dry weight of the ventricles should have been measured. As the heart was prioritised for histological staining (and drying the heart would have compromised this technique), an increase in the number of rats would have allowed for both histological and dry weight studies to occur.

c) The numbers of rats per group should have been increased in the Etn MI study, as the experiment was near to approaching an adequate power level for statistical significance in a few variables. The number of rats increased in the Etn hypertrophy study may have elicited significant results in the behaviour testing.

d) Due to mortality in the models and some technical complications with samples, the number of rats per group was not equal. This could have resulted in the occurance of a type II error, whereby a false negative result could have been possible. However, even with uneven numbers, the sample size in all groups was large enough to reach statistical significance.

e) Oxidative stress variations was expected to play a large role in this study due to results found in previous studies using ISO, Etn or Mg^{2+}. As such, oxidative stress should have been measured not only in the plasma but also in homogenised myocardium and neuronal cells. Measurement of scavenger enzymes could have also provided clarity to the model and interactions of Etn and Mg^{2+}.

f) A stain that distinguishes between connective tissue, muscle fibres, collagen, nuclei and cytoplasm (such as Masson's Trichrome stain) should have been used instead of the H&E stain. This stain would have highlighted the collagen depositions common to ISO administration and therefore allowed for easier and more accurate quantification of ISO-induced damage.

g) In humans, often MI occurs when individuals have pre-existing health problems. In the models of MI and hypertrophy used in this study, the rats were healthy before cardiac insult was induced. This makes the results difficult to extrapolate to human studies.

h) Throughout the year there were alterations to the rat housing facility in terms of lux rectification, temperature and housing organisation. This was unavoidable as the rat facility is constantly being upgraded to meet international standards. A constant environment was aspired to at all times.

i) It is possible that the physical condition of the rats due to a catecholamine overdose and the possibility of ISO affecting neurotransmitters may have affected the behaviour results and as such may have caused a false positive of depressive or anxious states.

j) A TTC-negative area is present in the control hearts. This is considered an artefact and may have arisen due to damage during heart extraction, perfusion or freeze-damage. The ISO model induces a global infarction, instead of a focal ischemic region which is common with other techniques such as coronary artery ligation. To account for any discrepancies, the TTC results in this study were analysed by two different observers and the same pattern of results were obtained.

k) Finally, in the hypertrophy model, left ventricular pressure should have been measured instead of arterial pressure. Due to technical problems with the equipment as well as financial constraints of the project, left ventricular pressure was unable to be measured.

5.6 Future Studies

The results of this study have brought into light many prospects for future studies. Besides the future studies mentioned in the discussion above, additional aspects should be considered.

a) The effects of ISO and Etn should be investigated on the left ventricle particularly in terms of chamber size. By taking coronal sections of the hearts and conducting histological tests on them, the question as to the type of hypertrophy induced by ISO and Etn may be answered. However, ensuring that each heart is excised at the exact same part of the cardiac cycle will provide some challenges.

b) Measurements of ECG and BP (tail cuff measurements) should be conducted on a conscious rat intermittently. This would provide information as to the effects of ISO, Etn and Mg^{2+} during disease progression.

c) Future studies should assess the cardiac output and pressure-volume loops of the heart using echocardiography. Due to financial constraints and limited access to the equipment, echocardiography was not possible in this study.

d) As the FST is being brought into contention recently as a test for depression, the study may elicit clearer results with the sucrose-preference test or the social interaction test.

e) Other mechanisms of ISO-induced behavioural disturbance should be investigated such as apoptosis (in both the brain and heart tissue), renin-angiotensin-aldosterone system functioning, mitochondrial functioning, cytokine expression (in the heart, brain and circulating in the blood) and electrolyte homeostasis (analysis of urine samples). Due to financial constraints, these parameters were not assessed in the current study.

f) As Etn is sourced exogenously from food and drink, a study should be conducted on the chronic administration of Etn in both MI and hypertrophy models. Pilot studies for this chronic Etn study were designed and carried out in our laboratory and the study is currently underway.

CONCLUSION

The results show that administration of ISO at 67 mg/kg as described by Arteaga de Murphy et al. (2002) elicited measurable MI with a low mortality rate. Further characterisation of this model revealed disruptions to the electrophysiology, haemodynamic and gross structural parameters of the heart. The model also induced systemic alterations such as increased oxidative stress, organ weight changes and modified loss of BW. Necrosis in this model could have occurred due to ISO-induced hypotension or oxidative stress. The characterisation provides the scientific community with a novel, low mortality model which can be used to test the cardiac and systemic effects of a multitude of substances. Characterisation of this model also allowed for the successful testing of therapeutic substances described in this study: Etn and Mg^{2+}, *in vivo*.

Etn lowers the mortality associated with the ISO-induced MI model. The mechanisms by which this occurred are ambiguous, yet it appeared that Etn altered the architecture of the heart to aid in compensation of the necrotic tissue. This architectural change may have occurred through a STAT-3 mediated pathway, as previously Etn has been shown to interact with STAT-3 (Fig. 38). The interaction of Etn with voltage-activated K^+ channels was not directly measured in this study but ECG parameters were affected by Etn administration, suggesting communication between Etn, electrolytes and ion channels (Fig. 38). This caused improvement to various ECG parameters by reduction of large Q- and T-waves, but also elicited some abnormal ECG activity such as altered QTc, shortened T_{peak}-T_{end} and decreased P- and S-wave amplitudes. Etn may also have interacted systemically with ion channels to accelerate fluid clearance in the lungs. The use of Etn as a cardiac-specific therapeutic agent should be taken with caution however, as pre-treatment did elicit some adverse effects on BW. However, further investigations are required to assess the impact of Etn on the appetite of the rat. Overall, the contribution of Etn as a protective agent is not only limited to the heart but also systemically.

The contribution of Mg^{2+} as a protective agent was also investigated and the results indicate that Mg^{2+} protects against ISO-induced hypotension in a model of MI. This may have been

modulated by the interaction of Mg^{2+} with the Ca^{2+} overload associated with ISO administration (Fig. 38). Systemically the kidneys were affected by Mg^{2+}. As the kidneys filter Mg^{2+} in an attempt to maintain homeostasis in the body, the alterations in kidneys/BW ratio does not confirm a negative or positive effect of Mg^{2+} therapy (Fig. 38). Although Mg^{2+} does not reduce infarct size or improve ECG characteristics (as was expected), there still remains a place for Mg^{2+} therapy in cardiovascular medicine as Mg^{2+} is an essential nutrient and a deficiency creates adverse effects on physiological functioning. Therefore the cardiovascular treatment of fatal arrhythmias and hypertension should still be dependant upon Mg^{2+} therapy.

The cardiovascular disruptions associated with cardiac hypertrophy, and the potential protective effects of Etn during cardiac remodelling were also investigated in this study. The interaction of Etn with the cell membrane requires further investigation as this may have been the mechanism by which Etn affected ECG parameters. The decrease in heart rate by Etn should be given much future attention, as this may be the result of an improved contractile state of the heart due to the increased HW/BW ratio (Fig. 38). The effects of Etn were also investigated on neurological functioning post-cardiac insult. It was expected that ISO-induced cardiac hypertrophy would cause alterations in the anxiety and depression state of the rat. The results of this study were not significant due to the small numbers of rats tested, however it was expected that Etn would improve affective states of the rats due to its interactions with renin, apoptosis, STAT-3 and cytokines, and electrolytes (Fig. 38) as previously shown by other studies.

This study has characterised a novel, low mortality model of ISO-induced MI that requires one single injection using a low dose of ISO. Figure 38 below summarises all results. It was also shown that Etn, a novel cardioprotective factor, reduced mortality possibly through augmentation of compensatory hypertrophy. Mg^{2+}, a long standing cardioprotective factor, reduced the hypotension associated with ISO; but did not protect against ISO-induced necrosis. This study also showed that in a model of cardiac hypertrophy, Etn again amplified the hypertrophic response. The evidence available in support of Etn's ability to alter behaviour requires further investigation with a larger sample size of rats.

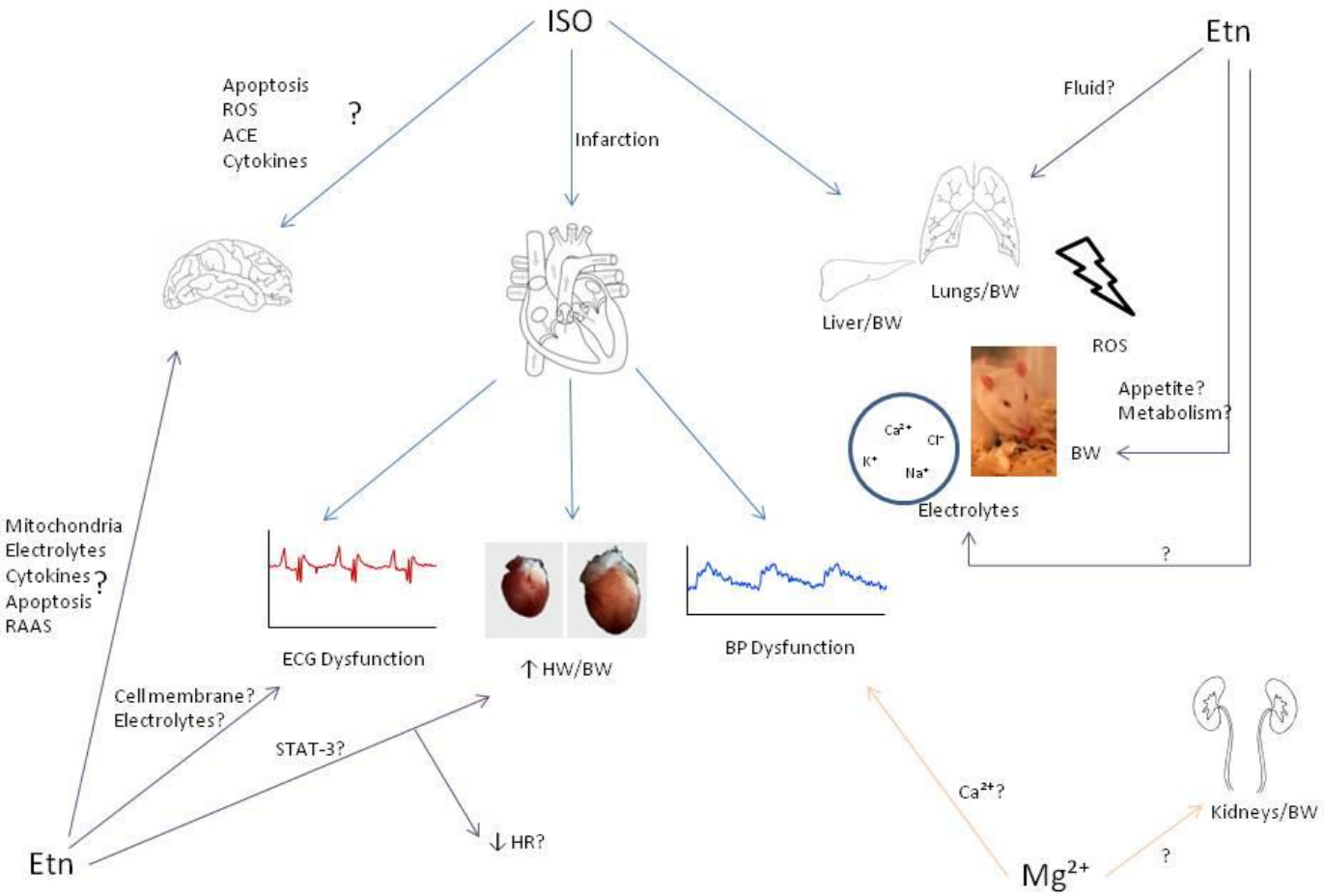


Figure 38: A summary of the results showing the effects of isoprenaline, ethanolamine and magnesium on cardiac and non-cardiac structures.

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PUBLICATIONS AND ABSTRACTS PRESENTED

Abstracts published in peer reviewed journals:

Garson C, Kelly-Laubscher RF, Bugarith K and Gwanyanya A (2012). Effects of magnesium administration on isoprenaline-inducd acute myocardial infarction in adult Wistar rats. *SA Heart Journal* Vol. 9 (3): 174-175

Garson C, Gwanyanya A, Bugarith K and Kelly-Laubscher RF (2012). The effects of acute ethanolamine administration on isoprenaline-induced myocardial infarction in adult Wistar rats. *SA Heart Journal* Vol. 9 (3): 174

Garson C, Gwanyanya A, Bugarith K and Kelly-Laubscher RF (2012). The effects of acute ethanolamine administration on isoprenaline-induced myocardial infarction in adult Wistar rats. *Journal of Molecular and Cellular Cardiology* Vol. 53 (2): S3

Work presented at conferences (national):

Garson C, Blackhurst DM, Bugarith K, Gwanyanya A and Kelly-Laubscher RF (2012). The effects of acute ethanolamine administration on isoprenaline-induced myocardial infarction in adult Wistar rats. *South African Heart Association*.

Garson C, Blackhurst DM, Bugarith K, Kelly-Laubscher RF and Gwanyanya A (2012). Effects of magnesium administration on isoprenaline-induced acute myocardial infarction in adult Wistar rats. *South African Heart Association*.

Work presented at conferences (international):

Garson C, Blackhurst DM, Bugarith K, Gwanyanya A and Kelly-Laubscher RF (2012). The effects of acute ethanolamine administration on isoprenaline-induced myocardial infarction in adult Wistar rats. *International Society for Heart Research, Canada*.

Papers awaiting publication:

Garson C, Gwanyanya A, Bugarith K and Kelly-Laubscher RF (2012). Characterisation of a low mortality isoprenaline-induced myocardial infarction model in adult Wistar rats. *Journal of Pharmacological and Toxicological Methods* (currently awaiting acceptance).

APPENDIX

9.1 TTC Stain from Defrosted Heart Sections

9.1.1 Recipe for TTC Buffer Solution A

100 M Monobasic sodium (acidic phosphate)	15.6 g
Distilled water	1000 ml

9.1.2 Recipe for TTC Buffer Solution B

100 µM Dibasic sodium (alkaline phosphate)	14.2 g
Distilled water	1000 ml

9.1.3 Recipe for 1% TTC Solution

Mix 4 parts solution B : 1 part solution A and titrate to pH 7.4

Add 250 mg TTC in 25ml buffer solution

TTC stain was conducted using the standard protocol

9.2 Infarct Size Quantification with ImageJ

File → Open → Scan picture: which displays heart sections of one rat per group for all 4 groups (this is the initial method of analysis)

For second method of analysis, view one heart at a time

View one side of the heart at one time

Zoom to accurate viewing

Select polygon icon

Hold shift and click along outline of heart slice

Release shift

Hold Ctrl and m

This will measure the entire area of all the slices cumulatively

Edit → Clear outside

Image → Colour → split channels

Close first window

Only use the “red” channel

Image → adjust → threshold

The grey is the infarcted area, but with ImageJ this cannot be measured so there is a need to measure the red area and minus it from the total in an excel worksheet.

Hold shift and click red part of the slices making sure to not include the white cut out areas (ventricles etc) or the grey infarcted area.

The Ctrl and m (or analyse → measure)

Open excel worksheet and input values from measure panel in Image J.

9.3 Haematoxylin and Eosin Stain for Frozen Sections

The recipe for solutions as well as the method for Mayer's haematoxylin was followed using the standard protocol (Bancroft and Gamble, 2008).

9.3.1 Recipe for Haematoxylin

Haematoxylin	1 g
Distilled water	1000 ml
Potassium or ammonium alum	50 g
Sodium iodate	0.2 g
Citric Acid	1 g
Chloral hydrate SLR	50 g

9.3.2 Recipe for Eosin

Eosin solutions are commercially available but an eosin solution can be made with the following recipe:

Eosin Y	2.5 g
Distilled water	495 ml
Glacial acetic acid	0.5 ml

9.3.3 Optimised Procedure for H&E Staining

Wash in running tap water	1 min
Place in hematoxylin	5 min
Wash in running tap water	1 min

Place in Scotts	30s
Wash in running tap water	30s
Place in eosin	30s
Wash in running tap water (manipulating)	15s

Place in alcohol solutions ranging from 96% to absolute

Stand in xylol	30s

Place entellan on coverslips for adhesive of cover slip to slide

Allow to dry	5 min

9.3.4 Quantification of Necrosis using ArcSoft Photo Studio Software

Open all 15 sections of one heart at a time

Use wand and click white area

Click 'delete'

File – save as – desktop

Next section

When all ventricular spaces have been deleted to appear white use photos in ImageJ

9.3.5 Quantification of Necrosis using ImageJ Software

Line tool, draw over 200µm line

Analyse – set scale – change to 200µm – tick global

CTL A – CTL M (this gives total value of picture)

Magic wand – highlight white spaces of ventricle (shift click will highlight multiple areas)

CTL M – (this gives total value of white spaces in µm)

Process – subtract background - enter

Plugins – colour deconvolution – H&E Roisin – enter

Use colour 1 (close others)

Image – adjust – threshold

Analyse – set measurements – tick 'area' and 'limit to threshold'

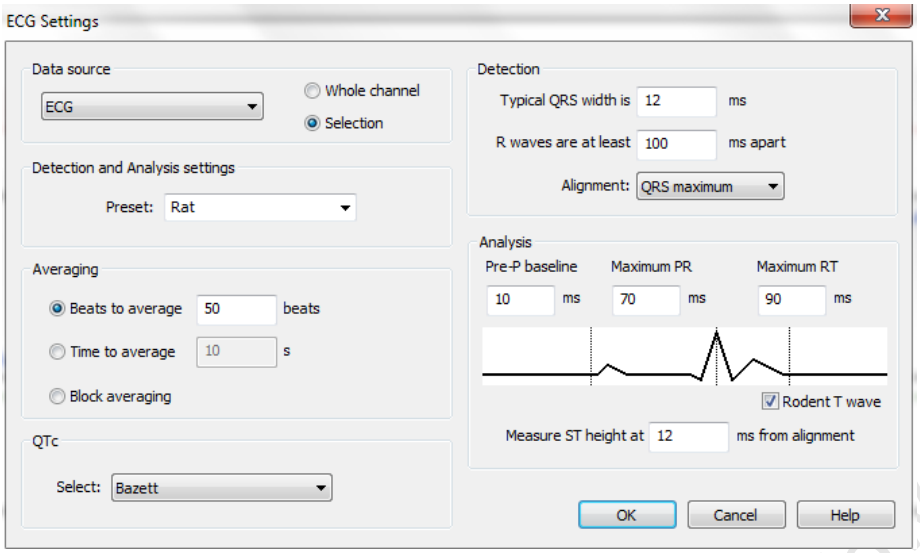
Adjust threshold to suit picture

CTL M (this give the area of the red)

Transfer to excel data sheet

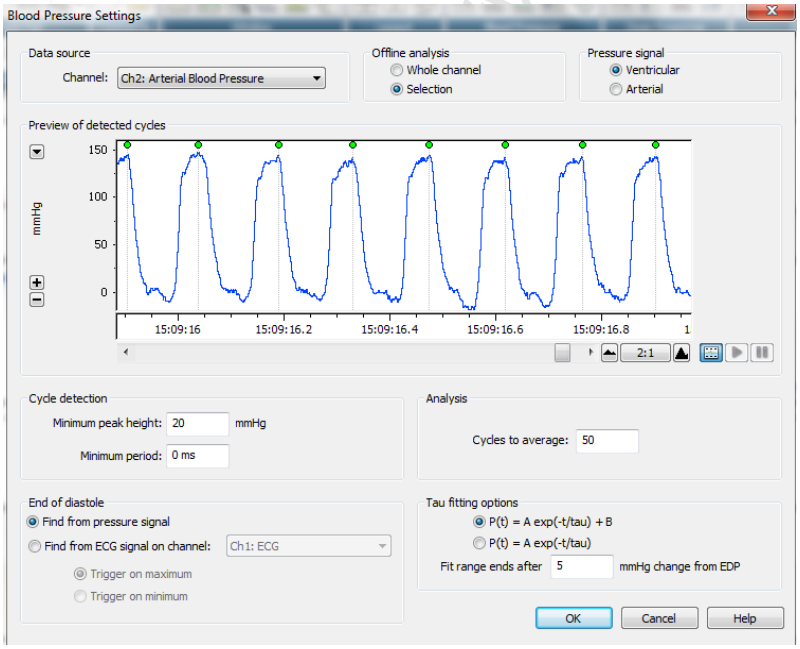
For necrotic area use formula: whole cell minus healthy cell divided by whole cell

9.4 ECG Settings in ECG Analysis Module of LabChart Pro



The ECG was analysed with a rat preset. The Bazett formula was chosen to calculate the QTc (See Discussion 5.2.2.3). The detection of the typical QRS-segment width was set to 12 ms and the R-waves were set to at least 100 ms apart. The graph was checked for inclusion of a rodent T-wave.

9.5 BP Settings in BP Analysis Module of LabChart Pro



The analysis was type was selected for either ventricular or arterial BP. For both parameters, the cycle detection was set at 20 mmHg with a minimum period of 0 ms. For left ventricular

pressure, the relaxation time constant (Tau) formula coined by Weiss et al. (1976) was used.

Equation: P(t) = A exp(-t/tau) + B.

9.6 *EPM and OF Settings for Noldus

Dynamic subtraction detection

Sample rate: 5.000

Brightness/contrast: current weight = 11, contrast = 29 to 121

Centre point detection

*These values and settings will vary per experimental room/environment. All behaviour experiments in this study were conducted in the exact same environmental conditions.

9.7 FST Criteria for Movement Classifications

9.7.1 Climbing

Usually involves the movement of all for limbs

The head is tilted backwards/upwards direction

The front paws are touching the cylinder

The body is aligned parallel to the cylinder

9.7.2 Swimming

Both the rear limbs are involved

The body is aligned perpendicular to the cylinder

The legs move faster than when the rat floats and the tail is often involved

Swimming includes changing direction, diving, head shaking and wiping ears

9.7.3Floating (Immobility)

The rat appears immobile

The rear limbs move very slowly with no involvement of the front limbs

Occasional twitching of the leg to stay afloat

9.8 Microscope and Camera Settings

Microscope set to Reel 3, IH, eyepiece 0

Camera settings: RGB, 1300 x 1030, standard colour

Images saved as ".tif" files

9.9 Exclusion of Rats from Study

Group		ISO Model		Etn MI				Mg MI				Etn Hypertrophy				
		Control	Disease	Control	ISO	ISO + Etn	Etn	Control	ISO	ISO + Mg	Mg	Control	ISO	ISO + Etn	Etn
Death		0	6	0	6	1	0	0	0	0	0	0	5	4	0
Exclusion of rat by heart rate		0	0	0	0	0	1	0	0	1	0	0	0	0	0
Exclusion of rat by BP		0	0	0	0	0	0	0	1	0	0	0	0	0	0
Culled		0	0	0	0	0	0	0	0	0	0	1	0	0	1

9.10 Appendix References

Bancroft, J. D., & Gamble, M. (2008). Theory and practice of histological techniques Elsevier Health Sciences.

Weiss, J. L., Frederiksen, J. W., & Weisfeldt, M. L. (1976). Hemodynamic determinants of the time-course of fall in canine left ventricular pressure. *Journal of Clinical Investigation, 58*(3), 751.