Cardiac Injury during Kainic Acid Induced Seizures: Effects of Gender on ECG

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**Abstract**

Epilepsy is associated with an increased mortality rate. In 7 to 17% of epileptics, the cause of death is unknown and this has been termed sudden unexplained death in epilepsy (SUDEP). Seizure-induced cardiac changes have been implicated in the pathophysiology of SUDEP and impaired autonomic regulation is not uncommon in epilepsy. Epileptic patients generally present with a higher heart rate than healthy individuals and tachycardia is frequently reported during seizure activity. Ictal bradycardia is rare, occurring in ~2% of seizures. It is hypothesised that seizures result in a surge of sympathetic activity which leads to tachycardia and heart damage. SUDEP is more prevalent in males than females and oestrogen is associated with cardio- and neuro-protective effects. Therefore it is expected that seizure severity, arrhythmias and cardiac pathology will be higher in males. In this study, kainic acid (KA, 10 mg/kg, s.c.) was used to induce seizures in male and female Sprague-Dawley rats. Electrocardiograph (ECG), electrocorticograph (ECoG) and behavioural data were recorded simultaneously for 3 hours post KA administration. KA administration resulted in a rapid significant decrease in heart rate (>30%) which lasted for 30-60 min. Bradycardia was associated with hypoactivity and low seizure behaviours. At 100 min post KA dosing, seizure severity increased resulting in an elevated cumulative behavioural seizure score (Level 3+) in conjunction with an increase in heart rate (20%). Wet dog shakes were the most commonly observed behaviour in males while females exhibited higher seizure behaviours such as myoclonic jerks, foaming and rearing. Tonic-clonic seizures were only observed in female KA animals. ECoG traces were analysed by Fast Fourier Transformation of different frequency bands. Females showed greater increases in power across all ECoG frequency bands. The greatest increase occurred in the theta band (4.75-6.75 Hz) which is associated with hippocampal activity. The P wave showed the largest ECG change during seizure activity. In males the P wave duration significantly decreased by 30-40% while no change was observed in females. In females significant decreases in P wave amplitude of up to 50% were observed while no significant changes occurred in males following KA administration. A prolonged PR interval was also present in females during bradycardia and tachycardia. Gender specific alterations in the QTc interval were also observed, with QTc prolongation occurring in males and QTc shortening in females. Histological examination of apical and mid-plane myocardium found that KA induced seizures resulted in left ventricular pathology observed by the presentation of oedema, inflammatory cell infiltration, intra-cardiac haemorrhage and myofibril vacuolisation 48 hours post KA. The results from this study demonstrate that KA induced seizures were associated with altered cardiac activity as
observed by heart rate and ECG changes. Seizure induced tachycardia may predispose the subject to fatal arrhythmias and myocardial damage, whereas bradycardia leading to asystole could also be implicated in SUDEP. Gender differences were also apparent with females exhibiting more severe seizure activity and greater alterations in ECG variables which is in contrast to preliminary clinical data. The findings from this study suggest that either testosterone has a protective effect or oestrogen may have pro-convulsant effects which enhance seizure induced changes.
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3α-diol: 3α-androstanediol
ACh: Acetylcholine
AED: Antiepileptic Drug
AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANS: Autonomic Nervous System
AV: Atrioventricular
AVOVA: Analysis of variance
BPM: Beats per minute
BP: Blood Pressure
Ca2+: Calcium Ion
cAMP: cyclic Adenosine Monophosphate
Cl−: Chloride Ion
CNS: Central Nervous System
DHT: 5α-dihydrotestosterone
DMH: Dorsomedial hypothalamus
DNQX: Dinitroquinoxaline
ECG: Electrocardiogram
ECoG: Electrocorticograph
EEG: Electroencephalogram
ER: Oestrogen Receptor
GABA: γ-Aminobutyric acid
GAD: Glutamic Acid Decarboxylase
GPCR: G-Protein Coupled Receptor
GTC: Generalised Tonic-Clonic
H & E: Haemotoxylin and Eosin
HR: Heart Rate
<table>
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<th>Abbreviation</th>
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<tr>
<td>IML</td>
<td>Intermediolateral</td>
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<tr>
<td>K+</td>
<td>Potassium Ion</td>
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<tr>
<td>KA</td>
<td>Kainic Acid</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium Chloride</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>Na+</td>
<td>Sodium Ion</td>
</tr>
<tr>
<td>NBF</td>
<td>Neutralized Buffer Formalin</td>
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<tr>
<td>NMDA</td>
<td>N-Methyl-D-aspartic acid</td>
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<td>NO</td>
<td>Nitric Oxide</td>
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<td>NTS</td>
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<td>PNS</td>
<td>Parasympathetic Nervous System</td>
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<td>PSD</td>
<td>Power Spectrum Density</td>
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<tr>
<td>QTc Interval</td>
<td>Corrected QT interval</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>SA</td>
<td>Sinoatrial</td>
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<tr>
<td>SE</td>
<td>Status epilepticus</td>
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<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<td>SNS</td>
<td>Sympathetic Nervous System</td>
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<td>SUDEP</td>
<td>Sudden Unexplained Death in Epilepsy</td>
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<td>TLE</td>
<td>Temporal Lobe Epilepsy</td>
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<td>VGCC</td>
<td>Voltage Gated Ca2+ Channels</td>
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<td>WDS</td>
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INTRODUCTION

1.1 EPILEPSY

1.1.1 Epilepsy Causes

Epilepsy affects approximately 50 million people worldwide with a prevalence of 8.2 per 1,000, although this figure is estimated to be higher in developing countries. Epilepsy is associated with an increase in mortality rate of two to three times that of the general population (WHO, 2001). Epilepsy is defined as a condition characterised by recurrent epileptic seizures, unprovoked by any immediate identified cause (Banerjee et al., 2009). In 50 to 60% of patients the underlying pathology is unknown (Engelbourghs et al., 2000). Factors which predispose patients to epilepsy include traumatic brain injuries, brain tumours, central nervous system (CNS) infections, cerebrovascular disease and neurodegenerative disorders (Annegers et al., 1996). Perinatal injury or hypoxia, as well as developmental disorders can initiate seizures in children (WHO, 2001). There is a 2.5-fold increase in the risk of developing epilepsy in individuals who have a first-degree relative with epilepsy (Annegers et al., 1996).

1.1.2 Pathophysiology

Epileptic seizures are initiated by a persistent increase in neuronal excitability due to an imbalance between excitatory and inhibitory processes in the brain (Engelbourghs et al., 2000). The onset of a seizure occurs when a group of neurons becoming hyperexcitable. These neurons undergo prolonged depolarisation associated with rapid firing of repeated action potentials. This discharge can result in the recruitment of adjacent neurons and spreading of electrical activity to other areas of the brain (Engelbourghs et al., 2000). Abnormal channel functioning, alterations in the neuronal ionic micro-environment and irregular neurotransmitter activity are proposed to be involved in ictogenic activity.

Seizures can be generated by genetic and environmental factors which alter the properties of the neuronal membrane. Genetic factors include mutations involving ion channels such as voltage-gated calcium (Ca^{2+})-, potassium (K^{+})- or sodium (Na^{+})-channels (Chapman, 2000). Environmental factors such as trauma, oxygen deprivation, tumours and infection can cause abnormal cellular discharges by altering ion levels or channel function (Engelbourghs et al., 2000). Epileptic neurons often have enhanced Ca^{2+} conductance due to increased efficacy or number of Ca^{2+} channels (Engelbourghs et al., 2000). Increased extracellular levels of K^{+} can also initiate seizure activity. The reduced outward K^{+} current causes depolarisation of the neuron, triggering Ca^{2+} influx and spike discharges (Dichter, 1997; Engelbourghs et al., 2000).
\(\gamma\)-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter. Decreased functioning of this system has been hypothesised to be involved in the pathophysiology of seizure generation. GABA receptors are subdivided into two main groups, ionotropic GABA\(\text{A}\) receptors and metabotropic GABA\(\text{B}\) receptors. GABA\(\text{A}\) receptors are pentameric ion channels permeable to chloride ions (Cl\(^-\)), when activated they decrease the threshold of the neuron (Henderson et al., 2006). Epilepsy is associated with a decrease GABA release, reduced activity of GABA\(\text{A}\) receptors and a decline in glutamic acid decarboxylase (GAD) activity (De Deyn and MacDonald, 1990; Engelbourghs et al., 2000).

Glutamate is also strongly implicated in seizure generation. Glutamate exerts its action via activation of ligand-gated cation channels, increasing sodium and calcium conductance (Chapman, 2000). At present no specific genetic mutation relating to enhanced glutamatergic function has been linked to human epilepsy, although elevated glutamate levels have been reported in epileptic patients (Van Gelder et al., 1980; Engelbourghs et al., 2000).

### 1.1.3 Seizure Types

The symptoms a patient experiences depends upon the brain regions affected. There are two main types of seizures, partial or generalised (Banerjeea et al., 2009). Partial seizures are seizures which occur in a localised brain region. If the patient experiences no loss of consciousness the seizure is characterised as a simple partial seizure. If consciousness is lost, then it is a complex partial seizure. (McNamara, 1994; WHO, 2001; Banerjeea et al., 2009). Generalised seizures involve epileptic activity of the whole brain. Consciousness is lost and patients generally experience convulsant behaviour. Generalised seizures can be further subdivided into absent, myoclonic, tonic-clonic, tonic and clonic symptoms (WHO, 2001; Banerjeea et al., 2009). In some patients partial seizures may spread to the whole brain resulting in a secondary generalised seizure (Banerjeea et al., 2009). SE occurs when a patient has frequent seizures with no recovery of consciousness in between. It is a dangerous state which can lead to brain damage or death (WHO, 2001).

### 1.1.4 Treatment

Treatment of epilepsy aims to decrease excitation or enhance inhibition. This is achieved through altering neurotransmitter levels or the functioning of ion channels. Treatment is successful in up to 70% of cases and in some of these cases therapy can be ceased within 5 years with no relapse (WHO, 2001).
The most commonly used class of antiepileptic drugs (AED) are sodium channel blockers such as carbamazepine, phenytoin and valproate. These are effective at treating seizures by reducing the influx of Na\(^+\). This increases the threshold of the neuron which prevents hyperpolarisation (Engelbourghs et al., 2000). This class of drugs can have potential cardiac side effects by altering the activity of cardiac sodium channels, resulting in drug induced arrhythmias.

Drugs which enhance GABAergic transmission are effective for treating epilepsy. Vigabatrin blocks the action of GABA transaminase which inhibits the breakdown of GABA at the synapse. Benzodiazepines and barbiturates act to allosterically enhance the activity of the GABA\(_A\) receptors, resulting in enhanced Cl\(^-\) conductance (Engelbourghs et al., 2000).

At present there are few effective glutamate receptor antagonists on the market due to neurological side effects. Felbamate is a non-competitive antagonist of NMDA glutamate receptors which acts at the glycine binding site. Glutamate release can be inhibited using lamotrigine which also acts to inhibit Na\(^+\) channels (Engelbourghs et al., 2000).

1.2 SUDDEN UNEXPLAINED DEATH IN EPILEPSY

1.2.1 Definition

Epilepsy is associated with an increased mortality rate compared to the general population (Lhatoo et al., 2001). In 7 to 17% of epileptics the cause of death is unknown. This has been termed sudden unexplained death in epilepsy (SUDEP) (Sperling, 2001; Opherk et al., 2002; Evrengul et al., 2005; Schuele et al., 2007). SUDEP is defined as “sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death in patients with epilepsy, with or without evidence of a seizure and excluding documented SE, in which post-mortem examination does not reveal a toxicological or anatomic cause for death” (Nashef, 1997). ‘Definite SUDEP’ must meet all the criteria including post-mortem examination which does not establish a cause of death, whereas ‘probable SUDEP’ meets the criteria without an autopsy (Annegers et al., 1998; Sperling, 2001).

1.2.2 Risk Factors

Many risk factors have been reported which lead to an increased chance of SUDEP. These include high seizure frequency, seizure clusters, occurrence of generalised tonic-clonic (GTC) seizures, male gender, young age, subtherapeutic concentrations of AEDs, early onset of epilepsy, long duration of epilepsy and treatment with three or more AEDs (Leestma et al., 1989; Shorvon, 1997; Kloster and Engelskjon, 1999; Nilsson et al., 1999; Walczak et al., 2001;
Opeskin and Berkovic, 2003; Rugg-Gunn et al., 2004; Monte et al., 2007). There is a progressive increase in the risk of developing SUDEP with increasing seizure frequency. Seizure duration of greater than 30 years, 50 seizures of any type per month or three tonic-clonic seizures per year are associated with an increased risk of SUDEP (Walczak et al., 2001). If patients have more than 50 seizures in a year their risk of developing SUDEP increases by 10% compared to those who only had 2 seizures a year (Nilsson et al., 1999). However no common risk factor has been found in all SUDEP cases.

1.2.3 Pathophysiology

At present the pathophysiology of SUDEP is unknown. Impaired autonomic regulation and seizure induced cardiac changes have been implicated in its cause (Jansen and Lagae, 2010). Potential mechanisms of SUDEP include cardiac arrhythmias, autonomic imbalance, hypoxia, arrhythmogenic drugs and apnoea (Stollerberger and Finsterer, 2004).

Neuropathological changes such as decreased brain weights, cerebral oedema and structural lesions have been reported in 27 to 70% of cases (Kloster and Engelskjon, 1999; Langan and Nashef, 2003). Using brain magnetic resonance imaging (MRI) data, Garcia et al. (2001) detected morphological alterations in 25 of 37 SUDEP cases. Pathological changes included cerebral neoplasma, gray matter hypertrophy and hippocampal sclerosis. Pulmonary oedema and respiratory depression leading to apnoea is also commonly reported during seizures in humans and animals (Johnston et al., 1995; Johnston et al., 1997; Kloster and Engelskjon, 1999; Langan and Nashef, 2003; Ryvlin et al., 2006; Jansen and Lagae, 2010).

Cardiac abnormalities, including post mortem reports of myocardial ischaemia, seizure-induced QT-lengthening and interictal changes in heart rate variability have been described in patients with epilepsy (Ryvlin et al., 2006). Repetitive seizure-induced activation of the central autonomic nervous system (ANS) can lead to structural heart damage, increasing the hearts’ susceptibility to cardiac arrhythmias or ischaemia (Kloster and Engelskjon, 1999; Jansen and Lagae, 2010). Natelson et al. (1998) found pathological changes present in five out of seven hearts from epileptic patients. Four hearts had evidence of irreversible perivascular and interstitial fibrosis and all had reversible myocyte vacuolisation, predominantly in the subendocardium. Kloster and Engelskjon (1999) reported that 33% of SUDEP patients had non-fatal pathological changes. These post-mortem reports commonly indicated the presence of fibrosis of the atrophicventricular bundle or diffusely located in the myocardium.
The pathological evidence suggests that SUDEP is caused by autonomic dysfunction which leads to structural heart damage and cerebral and respiratory oedema. Compromised autonomic function in patients with epilepsy may predispose patients to sudden cardiac death.

1.3 KAINIC ACID-INDUCED EXCITOTOXICITY

1.3.1 Glutamate

Glutamate is the main excitatory neurotransmitter in the CNS. Its effects are mediated through the activation of ionotropic and metabotropic receptors. Ionotropic glutamate receptors are ligand-gated cation channels which mediate fast excitatory neurotransmission. These have been divided into three receptor subtypes, N-methyl-D-aspartate (NMDA), \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptors, based on agonist and antagonist binding studies (Bettler and Mullet, 1995; Castillo et al., 1997).

1.3.2 Glutamate Receptors

NMDA receptors are hetero-tetrameric receptors which are non-selectively permeable to cations. A unique property of NMDA receptors is their voltage activation. The channel is blocked by \(\text{Mg}^+\) ions which allow for a voltage dependent flow of \(\text{K}^+\) out of cell and an influx of \(\text{Ca}^{2+}\) and \(\text{Na}^+\) ions into the cell (McNamara, 1994).

AMPA receptors are highly expressed throughout the CNS (Bettler and Mullet, 1995). AMPA receptors are tetrameric cation channels made up of GluRI-4 subunits (Bettler and Mullet, 1995). The configuration of these subunits determines the permeability of the channel. A majority of AMPA receptors are impermeable to \(\text{Ca}^{2+}\) due to the presence of a GluR2 subunit. However AMPA receptors lacking the GluR2 subunit are highly permeable to \(\text{Ca}^{2+}\) and show fast desensitisation. These receptors are present in inhibitory interneurons of the neocortex and the hippocampus (Bettler and Mullet, 1995; Hestrin, 1993; Livsey et al., 1993; Bochet et al., 1994; Bleakman et al., 1998).

Kainate receptors are abundantly expressed in various brain regions, including the cerebellum, amygdala, hippocampus and spinal cord (Bettler and Mullet, 1995; Bleakman, 1999). They are involved in synaptic transmission, influencing both neuronal excitability and information transfer in the brain (Lerma, 2009). Kainate receptors are hetero- and homomeric ligand gated \(\text{Na}^+\) channels. GluR4-7 and KA1-2 subunits assemble to form kainate receptors (Bleakman, 1999). GluR5-7 can form functional homomeric ion channels. KA1 and KA2 subunits are able to form heteromeric channels with GluR5 and GluR6 subunits (Hollmann and Heinemann, 1994).
Kainate receptors display rapid onset activation and desensitisation (Bleakman, 1999; Lerma, 2009). It has also been proposed that kainate receptors have a modulatory role at the synapse by presynaptically controlling neurotransmitter release, particularly in the hippocampus (Lerma, 2009; Bettler and Mullet, 1995). Activation of kainate receptors decreases acetylcholine (ACh) binding to muscarinic receptors in the rat forebrain (Jin et al., 2000). Stimulation of presynaptic kainate receptors enhances GABA release at hippocampal interneuron connections which inhibits GABA release at interneuron–pyramidal cell synapses (Dakshinamurti et al., 1991; Lerma, 2009).

1.3.3 Kainic Acid

Kainic acid (KA; 2-carboxy-4-isopropenylpyrrolidin-3-ylacetic acid) is a neuroexcitant derived from the red alga Diginea simplex (Coyle, 1987; Jane et al., 2009). It is a rigid analogue of L-glutamate which binds to AMPA and kainate receptors (Bleakman and Lodge, 1998). KA is 30-fold more neurotoxic than glutamate (Bleakman and Lodge, 1998; Shero et al., 1998; Jane et al., 2009). It has a 5 to 30 times higher affinity for kainate receptors than AMPA receptors (Wang et al., 2005; Lerma, 2009). Kainate GluR5 and GluR6-type receptors rapidly desensitise in the continuous presence of KA, while AMPA receptors show little desensitisation to KA (Bettler and Mullet, 1995). KA1 and KA2 subunits show a higher affinity for KA than GluR5-7 receptors (Bleakman, 1999).

1.3.4 Excitotoxicity

Glutamate induced excitotoxicity is a mechanism of cell death in many neurological disorders including ischaemia, brain/spinal cord injuries, epilepsy, Alzheimer’s disease and Parkinson’s disease (Bettler and Mullet, 1995; Chen et al., 2002; Gleeson et al., 2010). It is triggered by the enhanced activation of metabotropic and ionotropic glutamate receptors (Lerma, 2009). KA mimics the endogenous excitatory amino acid, glutamate (Shero et al., 1998). It produces excitotoxic cell death through potent activation of AMPA and kainate receptors (Wang et al., 2005).

Binding of KA to AMPA and kainate receptors produces a robust influx of Na\(^+\) which strongly depolarises the cell, triggering the opening of NMDA and voltage gated Ca\(^{2+}\) channels (VGCC). This large influx of Ca\(^{2+}\) ions initiates many biochemical reactions, including reactive oxygen species (ROS) production and activation of Ca\(^{2+}\)-dependent proteases, protein kinases, phospholipases and nucleases. These lead to mitochondrial dysfunction and apoptosis (Bettler and Mullet, 1995; Wang et al., 2005). Enhanced inflammatory responses, hypertrophy of
astrocytes and microglia-macrophage activation also occurs which can result in further brain
damage (Wang et al., 2005; Epsztein et al., 2009). Changes in electrolye levels may also lead to
neuronal cell death due to osmotic stress and cell lysis (Simon, 2009). KA administration is
associated with elevated free radical species which can cause cellular damage. Milatovic et al.
(2002) found that KA (15 mg/kg, s.c.) induced seizures resulted in an increase in neuronal nitric
oxide (NO) levels. An increased of 633%, 314% and 365%, occurred in the cortex, amygdala
and hippocampus, respectively, at 90 min. This suggested that KA induced seizures produce
enhance free radical NO production which causes oxidative stress and depletion of energy stores.
The most sensitive brain regions to KA induced excitotoxicity are the hippocampal CA1 and
CA3 subregions and in the hilus of dentate gyrus (Wang et al., 2005). These areas are rich in
high-affinity kainate receptors and Ca$^{2+}$ permeable AMPA receptors (Brorson et al., 1997;
Lerma, 2009).

1.3.5 Model of Temporal Lobe Epilepsy

Temporal lobe epilepsy (TLE) is the most prevalent type of epilepsy (Sevcencu and Struijk,
2010). It accounts for 30 to 40% of epilepsies and is often unresponsive to pharmacological
management (Epsztein et al., 2009). Hippocampal sclerosis is the most common pathology
associated with TLE. Neuronal loss leads to reorganisation of hippocampal axonal connections
(Veliskova et al., 2000). The degeneration of pyramidal cells and interneurons leads to a
shrinking of the hippocampus (Epsztein et al., 2009). These changes result in enhanced
hippocampal excitability and manifestation of epileptic seizures (Simon, 2009).

Systemic or intracerebral administration of KA is used as an animal model of TLE (Dernovsek
and Sket, 1998; Epsztein et al., 2009). The most common pathological changes observed after
KA-induced seizures are degeneration of the entorhinal and piriform complex, amygdaloid
complex, hippocampal formation, bulbus olfactorius, tuberculum olfactorium and thalamic
nuclei (Ben-Air, 1985; Sperk, 1994).

KA induced seizures are initiated in the hippocampus. The hippocampus is the most sensitive
structure to the convulsant effect of KA due to the high concentration of high affinity KA
binding sites (Lothman and Collins, 1981; Ben-Air, 1985; Wisden and Seeburg, 1993). KA
induces prolonged depolarisation of hippocampal neurons resulting in epileptic discharge
(Bleakman and Lodge, 1998; Epsztein et al., 2009). It produces inflammation and apoptosis in
the hippocampus, resulting in neuronal loss (Gleeson et al., 2010). KA administration in rats
results in seizure induced death of CA3 and eventually CA1 pyramidal cells (Epsztein et al.,
This is because CA3 pyramidal cells are 30 times more sensitive to KA than CA1 pyramidal cells (Castillo et al., 1997).

KA’s neurotoxic effect is enhanced by its ability to reduce inhibition which leads to the generation of recurrent epileptic spikes (Lerma, 2009). This is hypothesised to occur due to degeneration of interneurons within the hilus, which results in a decreased number of GABAergic terminals (Epsztein et al., 2009).

KA alters behavioural responses and induces generalised seizures in rodents, observed as increased locomotor and rearing activity (Chen et al., 2002). Following administration of KA the animals exhibited staring behaviours and decreased responsiveness to stimuli before developing limbic seizures (Lothman and Collins, 1981).

### 1.4 ELECTROCARDIOGRAPH

#### 1.4.1 Physiology and anatomy of the Heart

Coordinated contraction and relaxation of the heart is critical to ensuring that blood is supplied to the body. The sinoatrial (SA) node is located in the right atrial epicardium, near the opening of the superior vena cava (Kubdu et al., 2000). The SA node is the pacemaker of the heart. It initiates the impulses which depolarise the rest of the heart and produce contraction (Catalano, 2002). Following the initiation of the heart beat at the SA node, the impulse is propagated along the internodal tracts and intra-atrial tract to activate the atrioventricular (AV) node (Catalano, 2002). The AV node is located at the base of the right atria. It acts to delay the excitation from the SA node to allow the atria enough time to completely relax and fill the ventricles (Catalano, 2002). The bundle of His and Purkinje fibres carry the impulse from the AV node to the ventricles, allowing the ventricles time to contract (Kubdu et al., 2000).

#### 1.4.2 Electrocardiograph

The electrocardiogram (ECG) is used to assess cardiac function. It is a graphical representation of the electrical changes which occur within the entire heart over one heart beat (Figure 1.1)

The P wave is the first upward deflection on an ECG. It represents the discharge of the SA node and the contraction of the atria (Kubdu et al., 2000) (Stouffee, 2009). The P wave represents depolarisation of the right and left atria (Stouffee, 2009). Factors which prolong impulse propagation in the atria (such as fibrosis or hypertrophy) will lengthen the duration of the P wave (Stouffee, 2009).
The PR interval represents the time it takes to conduct the excitation from the atria, through the bundle branches to the Purkinje fibres (Catalano 2002). It is used to measure the conductance time through the AV node. A long PR Interval or bundle branch block suggests conduction system disease (Marsh et al., 2008). The PR interval can be prolonged by factors which slow conductance through the AV node, such as fibrosis of the node, increased vagal tone or medication such as beta or Ca\(^{2+}\) blockers. It can be shortened when impulses reach the ventricles via a bypass tract to cause the ventricles to pre-excite (Stouffee, 2009).

The QRS complex represents the depolarisation of the ventricles (Catalano, 2002). Depolarisation of both ventricles occurs simultaneously. An abnormal QRS complex can occur due to tissue damage or electrolyte imbalances (Stouffee, 2009). Prolonged QRS duration can occur due to myocardial infarction (MI), hypokalemia, severe bradycardia or His-Purkinji system disease (Marsh et al., 2008; Stouffee, 2009). Increased QRS amplitude can be caused by decreased pericardial fluid or structural damage such as left or right ventricular hypertrophy or hypertrophic cardiomyopathy (Marsh et al., 2008; Stouffee, 2009).

The T wave of the ECG is the final wave and it corresponds to the relaxation of the ventricles (Kubdu et al., 2000; Catalano, 2002).

In the rodent ECG there is an absence of an isoelectric interval between the S wave and the T wave (Figure 1.1). This is due to different ventricle repolarisation mechanisms. In humans opening of potassium channels, such as the inward K+ rectifier and delayed K+ rectifier currents, are responsible for repolarisation of the myocardium (Keating and Sanguinetti, 2001). However in rodents, the primary current involved in repolarisation is the rapidly activating and inactivating 4-aminopyridine-sensitive transient outward current (Gussak et al., 2000; Antzelevitch, 2006).

![Figure 1.1: ECG for a single heart beat. Top image shows a human ECG trace and the bottom image represents a rat ECG trace with altered T wave.](image)
1.5 AUTONOMIC NERVOUS SYSTEM

1.5.1 Autonomic nervous system

The autonomic nervous system (ANS) maintains homeostasis through regulation of heart rate (HR), respiration, digestion, micturition and reproduction (Jansen, 2010). It is controlled by medullary reflexes and the cerebral cortex (Jansen, 2010). The ANS consists of two main subsystems, the sympathetic and parasympathetic nervous systems. Most viscera and organs have dual innervations with both nervous systems (Shields, 1993). The interaction between these systems is complex. Both systems are tonically active and operate in conjunction with each other (Iversen et al., 2000). These pathways generally have antagonising effects which act to control and maintain homeostasis between vital organs of the body such as the heart, lungs, liver and kidneys (Figure 1.2).

1.5.2 Sympathetic Nervous System

The sympathetic nervous system (SNS) is involved in the “fight or flight” response. It increases arousal and energy production while inhibiting gastrointestinal function. Activation of the SNS results in increased blood flow to lungs and skeletal muscle, increased HR and respiration and increased renin release (Janig and McLachlan, 1992; Shields, 1993; Vaseghi and Shivkumar, 2008). The main neurotransmitter involved in mediating sympathetic control is noradrenaline (NA). It is biosynthesised from tyrosine and neuronal stimulation leads to NA release by exocytosis (Vaseghi and Shivkumar, 2008). A unique feature of the SNS is its innervation of the adrenal medulla (Figure 1.2). Preganglionic fibres travelling in the splanchnic nerves directly innervate the adrenal glands (Shields, 1993). Sympathetic stimulation causes secretion of NA and adrenaline directly into the bloodstream. This widespread sympathetic discharge allows the SNS to mediate pupil dilation, increased HR and contractility, bronchodilation, vasoconstriction and vasodilation of skeletal muscle arterioles (Shields, 1993).

NA mediates its effect by activating either α or β adrenoreceptors. Adrenoreceptors are G-protein coupled receptors (GPCR) which mediate inhibitory or excitatory effects. Alpha adrenoreceptors are divided into two main groupings; α1 and α2 receptors. Subtypes of β adrenoreceptors include β1, β2 and β3 receptors.

Activation of α1, β1 and β2 adrenoreceptors increases intracellular levels of cyclic adenosine monophosphate (cAMP) which stimulates protein kinases and elevates intracellular Ca²⁺ levels (Vaseghi and Shivkumar, 2008). α1 adrenoreceptors are responsible for relaxation of gastrointestinal smooth muscle, secretion of salivary and sweat glands. They have positive
inotropic effects and enhance metabolic processes such as glycogenolysis and gluconeogenesis (Shields, 1993). β1 adrenoreceptors are predominately associated with sympathetic control of the heart by causing positive inotropic and chronotropic effects. Activation of these receptors also mediates lipolysis of fat and renin release (Shields, 1993). β2 adrenoreceptors are responsible for the relaxation of smooth muscle in the bronchi, gut and vascular smooth muscle of skeletal muscle (Shields, 1993; Du et al., 1995). All these responses are important in preparing the enhanced activity.

α2 adrenoreceptors are generally presynaptic and produce inhibitory actions by decreasing the production of cAMP (Shields, 1993; Du et al., 1995). They are primarily located presynaptically at nerve terminals and act to decrease sympathetic stimulation by inhibiting NA release (Shields, 1993; Vaseghi and Shivkumar, 2008).

1.5.3 Parasympathetic Nervous System

Activation of the parasympathetic nervous system (PNS) promotes the “rest and digest” response. This results in dilation of blood vessels and increased motility in the gastrointestinal tract. It is also involved in salivation, micturition and pupil constriction (Janig and McLachlan, 1992). The PNS exerts inhibitory effects on the heart, decreasing HR and contractility (Vaseghi and Shivkumar, 2008). Unlike the SNS, the PNS ganglia are located in proximity to the target organ. The preganglionic fibres tend to be long and the postganglionic fibres short (Figure 1.2; Shields, 1993).

ACh is the neurotransmitter of the PNS. It is synthesised by acetylation of choline by choline transferase. ACh is stored in vesicles and is released by preganglionic or postganglionic parasympathetic stimulation. The effects of ACh are rapidly terminated by degradation of ACh by acetylcholinases (Vaseghi and Shivkumar, 2008). There are two classes of ACh receptors, muscarinic and nicotinic receptors which modulate excitatory or inhibitory effects of ACh in the periphery and CNS.

Nicotinic receptors are pentameric ligand gated cation channels which are located in the central and peripheral nervous system. In the periphery they are present at neuromuscular junctions and preganglionic neurons, where they mediate fast postsynaptic excitatory neurotransmission (Quarta et al., 2007). Nicotinic receptors in the CNS play an important role in modulating the function of multiple transmitter systems, including dopamine, GABA, serotonin and glutamate (Quik and Jeyarasasingam, 2000, Gotti et al., 2006).
In the CNS muscarinic receptors are implicated in a variety of central processes, including cognition, motor control, arousal, thermoregulation, mood and nocioception (Wess, 1996; Gerber et al., 2001; Bymaster et al., 2002). In the periphery, muscarinic receptors regulate smooth muscle contraction, glandular secretion and HR (Bymaster et al., 2002). There are five subtypes of muscarinic receptors, M1-5. These are all GPCRs which play a key role in the regulation of the parasympathetic system (Gerber et al., 2001; Bymaster et al., 2002).

M1, M3 and M5 receptors are expressed extensively on postsynaptic terminals where they modulate fast synaptic transmission and metabotropic functions (Bymaster et al., 1999). These receptors are positively coupled to the activation of phospholipase C. Phospholipase C cleaves membrane phospholipids to diacylglycerol and inositol-triphosphate which results in activation of protein kinases and Ca$^{2+}$ release from the sarcoplasmic reticulum and endoplasmic reticulum (Caulfield, 1993; Bymaster et al., 1999). M1 receptors are highly expressed in the CNS where they have a major role in memory and learning, regulation of cognition and psychosis (Fisher, 2008). M3 receptors are present in the smooth muscle of airways and the gastrointestinal system where they induce contraction and mucus secretion.

Figure 1.2: Autonomic nervous system ganglion. Adapted from Iverson et al. (2000)
M2 and M4 receptors act primarily as inhibitory autoreceptors or heteroreceptors on presynaptic terminals. Activation of these receptors leads to a decrease in cAMP levels thereby reducing in neurotransmitter release (Caulfield, 1993; Bymaster et al., 1999). M2 receptors are present in the ventricular myocardium where they act to decrease contraction of the heart (Janig and McLachlan, 1992; Vaseghi and Shivkumar, 2008).

1.5.4 Central Autonomic Nervous System Anatomy

Normal functioning of the ANS involves a complex interaction between many brain regions. The central ANS involves the cortical limbic areas, including the amygdala, anterior insula, anterior cingulate cortex and posterior orbitofrontal cortex (Devinsky, 2004). These areas are connected with subcortical regions such as the hypothalamus, periaqueductal grey, parabrachial region in the pons, nucleus of the solitary tract (NTS) and ventrolateral medulla (Shields, 1993; Devinsky, 2004; Leung et al., 2006; Jansen and Lagae, 2010; Jehi, 2010).

The hypothalamus maintains homeostasis by integrating the activity of ANS nuclei and modulating endocrine function. The hypothalamus exerts its effect via interaction with the pituitary. Descending pathways from the hypothalamus innervate the midbrain and reticulospinal pathways from pons and medulla to interneurons in the spinal cord which influence intermediolateral (IML) cells (Shields, 1993). The right insular cortex is thought to predominately regulate sympathetic tone, whereas the left insular cortex modulates vagal parasympathetic tone (Wittling et al., 1998; Devinsky, 2004). The parasympathetic neurons of the vagus nerves are located in the nucleus ambiguus and dorsal motor nucleus. Neurons in the nucleus ambiguus control visceral smooth muscle, whereas fibres originating from the dorsal motor nucleus are primarily secretomotor in function (Shields, 1993). The vagus nerve innervates the heart, pancreas, liver, kidney and gastrointestinal tract. It also innervates smooth muscle and glands of the neck and thorax (Shields, 1993).

1.5.5 Cardiospecific effects of the Autonomic Nervous System

Cardiac activity is mediated by the sympathetic and parasympathetic nervous systems. The activity of these is modulated by two main influences. Descending pathways from the cerebral cortex, hypothalamus and periaqueductal gray matter mediate cardiac ANS activity in response to internal and external stressors. Medullary reflexes also modulate heart activity in response to the activation of baroreceptors, cardiac receptors and chemoreceptors (Schuele et al., 2007).
1.5.5.1 Sympathetic Nervous System

The sympathetic system is constantly active. The cardiac conductance system receives tonic stimulation from the ventrolateral medulla and the IML cell column. This results in increased firing of the SA node and enhanced AV conductance, elevating HR (Schuele et al., 2007; Jansen and Lagae, 2010). Sympathetic stimulation enhances ventricular and atrial contraction, increasing cardiac output (Vaseghi and Shivkumar, 2008). Stimulation of β adrenoceptors in the myocardium leads to activation of L-type Ca^{2+} channels resulting in an influx of Ca^{2+} into the myocytes and a release of Ca^{2+} from the sarcoplasmic reticulum which causes contraction of the myocardium (Iversen et al., 2000; Vaseghi et al., 2008). β adrenoceptors also decrease the threshold of firing of the cardiac pacemaker cells in the SA node, thereby increasing HR (Iversen et al., 2000). These effects are further potentiated by the release of adrenaline from the adrenal medulla (Iversen et al., 2000).

1.5.5.2 Parasympathetic Nervous System

Parasympathetic effects on the heart are controlled by the left and right vagus nerves which originate in the medulla (Vaseghi and Shivkumar, 2008). The SA node and AV node are densely innervated by the parasympathetic system (Vaseghi and Shivkumar, 2008). The right vagus nerve mediates the decrease in HR while decreased AV conductance and ventricular excitability are controlled by the left vagus nerve (Schuele et al., 2007). The NTS is involved in modulating parasympathetic activity. It receives descending inputs from the hypothalamus and forebrain (Shields, 1993).

M2 receptors are the main ACh receptor present in the ventricular myocardium where they decrease contraction by reducing intracellular Ca^{2+} levels. M2 receptors in atrial and nodal tissue activate a G_{K} protein causing a K^{+} efflux by directly inducing K^{+} channel opening (Vaseghi and Shivkumar, 2008). This results in hyperpolarisation of the SA node and slows conductance through the AV node (Iversen et al., 2000). ACh also decreases HR by increasing the threshold of the pacemaker cells and reduces force of contraction by reducing the L-type Ca^{2+} current (Iversen et al., 2000).

1.5.4 Autonomic Nervous System and Seizures

Impaired autonomic regulation and cardiac arrhythmia’s in epileptic patients are associated with long-term morbidity and mortality (Perssons et al., 2005; Monte et al., 2007). Seizures which arise from or spread to areas in the central autonomic network can mimic or alter autonomic effects (Devinsky, 2004; Jansen and Lagae, 2010). Seizure activity generally results in enhanced
sympathetic stimulation such as tachycardia (HR >100 bpm), tachypnea, hypertension, pupil dilation, diaphoresis and facial flushing. This can lead to seizure-induced cardiovascular dysfunction, pulmonary oedema and postictal depression of autonomic respiratory reflexes which may contribute to SUDEP (Devinsky, 2004). Provini et al. (1999) reported that autonomic activation was a common symptom of nocturnal frontal lobe epilepsy with tachycardia occurring in 88% of seizures and changes in respiratory rhythm in 77% of the cases.

Ictal parasympathetic activity or sympathetic inhibition leads to symptoms such as increased salivation, gastric acid secretion, peristalsis, decreased heart (HR <40 bpm) and respiratory rates and reduced blood pressure (BP; Devinsky, 2004). These can go unrecognised and therefore may be under reported.

1.6 CARDIAC ABNORMALITIES DURING SEIZURES

1.6.1 Ictal Tachycardia

Seizures have been associated with changes in HR. Symptoms of increased autonomic activation are frequently observed during seizures (Provini et al., 1999). Tachycardia is most commonly reported, occurring in up to a 100% of seizures (Opherk et al., 2002; Surges et al., 2009; Jansen and Lagae, 2010). Ictal tachycardia appears to be more common in TLE compared to other seizure origins (Jansen and Lagae, 2010).

The increase in HR may precede the seizure by 0.7 to 49.3 sec (Jansen and Lagae, 2010). Zijlamans et al. (2002) analysed 81 patients with intractable epilepsy who had prolonged electroencephalogram (EEG) and ECG monitoring. An increase in HR by 10 beats/minute (bpm) occurred in 93% of patients. In 23% of seizures the HR increase preceded both the EEG and the clinical onset. Leutmezer et al. (2003) found that sinus tachycardia occurred in 85% of complex tonic-clonic seizures. Retrospective analysis of SUDEP cases has shown similar results. Nei et al. (2004) reported that 94% of SUDEP patients had sinus tachycardia during or shortly after seizures, with a mean maximum HR of 149 bpm. This was significantly higher than the control epileptic group.

The results from these experiments suggest that tachycardia due to increased sympathetic activity or decreased parasympathetic control occurs during seizures. Tachycardia leading to malignant ventricular tachycardia, ventricular fibrillation and long-term myocardial damage could contribute to sudden death (Jansen and Lagae, 2010).
1.6.2 Ictal Bradycardia

Ictal bradycardia is rarely reported, occurring in fewer than 2% of seizures (Nei et al. 2000; Leutmezer et al., 2003; Devinsky, 2004). The incidence of bradycardia may be under reported as most studies look at retrospective data. In an experiment where epileptic patients were implanted with an ECG recorder for 24 months, bradycardia (<40 bpm) was reported in 35% of patients (Rugg-Gunn et al., 2004). Permanent pacemakers were required in 21% of patients which suggests that bradycardia leading to asystole could be implicated in SUDEP.

Bradycardia is more prevalent in seizures of temporal or frontal lobe origin. It may be more frequent in males or patients with left-sided foci (Devinsky, 2004). Cortical stimulation of the left insular cortex and amygdala has been found to elicit bradycardia and bradyarrhythmia’s (Oppenheimer et al., 1991; Jansen and Lagae 2010).

Although bradycardia is uncommon it should not be dismissed as a main contributor to SUDEP. Bradycardia leading to asystole more than 10 sec could cause severe hypoxemia which could lead to a fatal decrease in cerebral oxygen supply and sudden death (Surges et al., 2009).

1.6.3 ECG Abnormalities

Cardiac arrhythmias are caused by malfunctioning of cardiac electrophysiology caused by abnormal channel functioning or tissue damage. ECG abnormalities have been reported in 35% of seizures and 72% of epileptic patients (Nei et al., 2000; Devinsky, 2004; Surges et al., 2009). These include atrial fibrillation, supraventricular tachycardia, ventricular premature depolarisation, branch block and first degree AV block (Nei et al., 2000; Devinsky, 2004). Most changes are benign (Opherk et al., 2002). However potentially serious changes (such as ST depression and T wave inversion) have been reported to occur in 6 to 13% of seizures and these are markers of cardiac ischaemia (Opherk et al., 2002; Devinsky, 2004). Approximately 40% of patients with refractory epilepsy have at least one rhythm or repolarisation abnormality during or shortly following a seizure (Devinsky, 2004).

It is important to be aware of ictal ECG abnormalities as these can help identify those at risk of sudden cardiac death. The presence of AV block, intraventricular conduction defects, QT prolongation and a resting HR of greater than 90 bpm, are markers of sudden cardiac death (Zipes and Wellens, 1998). ECG abnormalities, such as ST elevation/depression and T wave inversion, have been reported to occur in 44% of patients (Zijlamans et al., 2002). Long seizure duration increases the occurrence of ECG abnormalities.
Seizures are associated with enhanced autonomic function. Excessive autonomic stimulation can cause arrhythmias resulting in structural heart damage which increases the hearts susceptibility to cardiac arrhythmias or ischaemia (Opherk et al., 2002). Ventricular fibrillation is the most common terminal event for sudden cardiac death (Dasheiff, 1991).

1.7 GENDER DIFFERENCES

1.7.1 Gender differences

Male gender is a risk factor for SUDEP with a male predominance of 54 to 77% (Leestma et al., 1984; Leestma et al., 1989; Earnest et al., 1992; Nilsson et al., 1999; Opeskin and Berkovic, 2003). Although seizure frequency is similar between sexes, MRI data has suggested that there is less seizure-induced brain damage in females (Briellmann et al., 2000). TLE is more common in males than in females. TLE in females is often associated with a reproductive endocrine disorder and oestrogen replacement therapy is generally effective at controlling the seizures which suggests that oestrogen is protective against TLE (Veliskova et al., 2000; Veliskova, 2006).

Males show greater changes in ictal HR activity and ECG abnormalities than females (Kirchner et al., 2002). Zijlamans et al. (2002) reported that ECG abnormalities occurred in 53% of males compared to 39% of females with intractable epilepsy. Males appear to have a higher incidence of ictal asystole than females. In one study conducted in the United Kingdom four out of twenty epileptic patients suffered asystole requiring a permanent pacemaker implant and all four patients were men (Rugg-Gunn et al., 2004). Ictal asystole could constitute a major contributor to SUDEP in males.

Gender differences have also been observed in cardiac function. Men have a higher incidence of coronary heart disease, myocardial infarction and sudden death (Cavasin et al., 2003; Tsang et al., 2007). Approximately 75% of sudden cardiac deaths occur in men (Zipes and Wellens, 1998). Females have a lower HR variability than males potentially due to reduced sympathetic influence. This could explain why females have a lower incidence of atrial fibrillation and sudden cardiac death than males (Kirchner et al., 2002).

These gender specific effects are most likely due to the major sex hormones, testosterone and oestrogen. Following menopause women exhibit an increase in seizure frequency Ovulation has also been associated with a decrease in seizure rates (Veliskova, 2006; Veliskova, 2007). Oestrogen has been reported to decrease the activity of NMDA and VGCC (Veliskova, 2007). It enhances GABAergic transmission by increasing GABA release and production as well as up-regulating GABA receptors (Saleh and Connell, 2003). Oestrogen also decreases neuronal NOS
activity, has antioxidant properties and inhibits apoptosis (Veliskova, 2007). All these effects act to decrease seizure generation and protect neurons from damage due to hyper-excitability. Testosterone however has been reported to have negative effects in epilepsy. It increases seizure activity by decreasing GABA levels in various brain regions and is generally reported to have pro-apoptotic properties (Cavasin et al., 2003; Gray et al., 2005; Tsang et al., 2007).

1.7.2 Oestrogen

Oestrogen is a gonadal steroid hormone which is associated with female reproductive physiology (Dluzen and McDermott, 2000). Oestrogen is the primary hormone involved in the female reproductive system (Dluzen and McDermott, 2000). It is also present in males and is involved in cardiovascular and neuronal functioning. Oestrogen is beneficial in many neurological disorders such as schizophrenia, Parkinson’s disease and multiple sclerosis (Veliskova et al., 2000). It has a lipophillic, small molecular weight hormone which is able to cross the blood brain barrier and easily reach the neuronal tissue (Veliskova, 2006). Oestrogen affects a variety of neuronal areas. These include the basal forebrain cholinergic system, caudate-putamen, hippocampus, brain stem and spinal cord. These areas mediate the effect of oestrogen effect on mood, locomotion, pain and cognition (McEwen, 2002).

Oestrogen receptors (ERs) are ligand-activated transcription factors (Enmark 1999). Interaction of oestrogen with ER induces a conformational change in the receptor protein which causes dimerisation of the receptor complexes. These complexes interact with the oestrogen response element, which results in initiation of gene transcription (Zhou et al., 2001). The genes transcribed by the ER depend on the cell and the promoter present (Smith et al., 1997). There are two subtypes of ERs, ERα and ERβ (Kuiper et al., 1996). ERα is widely expressed throughout the body, whereas ERβ has a much more restricted distribution (Kuiper et al., 1996).

Both receptors are present in the CNS. Oestrogen acts to decrease glutamatergic transmission and enhance GABA mediated effects in the CNS (McEwen, 2002; Saleh and Connell, 2003). It also produces neuroprotective effects by inhibiting transcription of pro-apoptotic proteins such as Bcl-2 (Bourque et al., 2007; Kuroki et al., 2001). Binding of oestrogen to ER mediates transcription of protective neuronal growth factors such as neurotrophins, brain derived neurotrophic factor and nerve growth factor (Miranda et al., 1996; Son et al., 1999). Oestrogen also has antioxidant properties, which can reduce damage caused by neurotoxins (D’Astous et al., 2005).
Women generally have a lower risk of developing cardiovascular disease, however this benefit is lost following menopause (Du et al., 1995). This suggests that oestrogen may mediate this protective effect. Oestrogen has many cardioprotective effects. It stimulates vasodilation, increases levels of high density lipoproteins and reduces atherosclerotic plaque formation (Du et al., 1995). The antioxidant properties of oestrogen and ER-mediated transcription of growth factors and anti-apoptotic proteins reduces cardiac damage following an ischemic event. This may explain why SUDEP is less common in females.

1.7.3 Testosterone

Testosterone is an androgen steroidal hormone. It is primarily secreted from the testes in males and ovaries in females. Serum testosterone levels decline with age and this is associated with a decrease in muscle mass and bone density as well as a decline in cognitive function (Hammond et al., 2001; Gray et al., 2005). Testosterone has neuromodulatory effects. At present there are controversial results about its neuroprotective ability. Clinical data suggests that testosterone is protective against neurological disorders such as Alzheimer’s disease, depression and anxiety (Hammond et al., 2001; Gray et al., 2005). However it can also enhance excitotoxic damage, such as seizure activity, due to reduced inhibitory transmission (Gray et al., 2005).

Testosterone, like oestrogen, mediates its effects by genomic and non-genomic mechanisms. It binds to androgen receptors or can be converted to oestradiol by aromatase where its effects are mediated through ERs (Bolour and Braunstein, 2005). Binding of testosterone to androgen receptors can activate gene transcription or act through signal transduction (Hammond et al., 2001). Androgen receptors are distributed throughout the body. They have been identified in the preoptic area and in the hypothalamus (Bolour and Braunstein, 2005). Testosterone produces neuroprotective effects by stimulating neuronal growth and survival (Gray et al., 2005). Testosterone increases transcription of growth factors such as nerve growth factor and p75 growth factor (Hammond et al., 2001). There is controversial data about the effect of testosterone on apoptosis. Testosterone has been found to inhibit apoptosis in human neuronal cell lines (Hammond et al., 2001) but also produces dose-dependent increases in myocyte apoptosis (Cavasin et al., 2003; Tsang et al., 2007). It could be that testosterone has tissue specific effects with regard to apoptosis.

There is also a mixed opinion on whether testosterone is cardioprotective or not. In males there is an increased risk of coronary heart disease. Testosterone adversely affects plasma lipid and lipoprotein profiles resulting in elevated low density lipoprotein levels. It also promotes thrombus formation and cardiac hypertrophy. Men have a higher incidence of MI and sudden
cardiac death than pre-menopausal women (Cavasin et al., 2003; Tsang et al., 2007). Testosterone treatment enhances the activity of the renin-aldosterone-angiotensin system which leads to increased risk of hypertension in males (Tsang et al., 2007). However there is also evidence suggesting that testosterone has cardioprotective effects. It can decrease ischemic Ca$^{2+}$ overload, activate mitochondrial K$_{ATP}$ channel and increase responsiveness to NA (Crews and Khalil, 1999; Tsang et al., 2007). These effects can result in reduced cardiac damage and increase function post-MI. At present the mechanism of these effects are unknown, although it has been suggested that testosterone may act similar to oestrogen (Hammond et al., 2001).

1.8 HYPOTHESIS AND AIMS

These studies reveal evidence to suggest that changes in autonomic function could be implicated in SUDEP. Tachycardia due to enhanced sympathetic stimulation or decreased parasympathetic stimulation is the predominated effect observed during seizure activity. Although ictal bradycardia is rare, occurring in 2% of seizures it cannot be dismissed as a factor in SUDEP. Tachycardia could result in long-term myocardial damage, whereas bradycardia leading to asystole may be a main contributor to SUDEP.

It has been proposed that seizure activity results in a “sympathetic storm” caused by a surge in NA and adrenaline. This leads to ictal tachycardia, ECG abnormalities such as QT elongation and structural heart damage (Figure 1.2, blue arrow). Another possible mechanism which could lead to SUDEP is elevated feedback activation of the parasympathetic system. In susceptible individuals the feedback response may be too strong, resulting in fatal bradycardia and asystole (Figure 1.2, green arrow). However further research is required to support this.

Gender differences appear to play a key role in SUDEP. Males have a higher incidence of bradycardia, asystole and ECG abnormalities than females. Oestrogen has been reported to have cardio- and neuro- protective effects and these could explain the lower incidence of SUDEP in females. Gender differences in the autonomic system may play an important role in SUDEP. Males have more enhanced sympathetic activity compared to females. Females have heightened parasympathetic tone compared to males and therefore an enhanced ability to resist increases in sympathetic output (Saleh and Connell, 2000). If SUDEP is a result of enhanced sympathetic activity leading to cardiac damage or elevated feedback activation of the parasympathetic system, then oestrogen may act to reduce this feedback response (Figure 1.2, pink lines).

The aim of this study was to analyse ECG and electrocorticography (ECoG) data during seizure activity induced by systemic KA. KA induced seizures result in a rapid change in animal
behaviour with the development of seizure behaviours lasting up to three hours post KA administration. Therefore it was hypothesised that during this time period ECG activity would be altered, similar to that reported during clinical studies in epileptic patients. It was also expected that seizure activity would induce tachycardia and prolongation of the QT interval due to elevated sympathetic activity. Cardiac morphology was examined 48 hours following seizure induction to determine if cardiac damage occurs as a result of seizure induction. Gender was also investigated to determine if sex has an effect on seizure behaviours as well as HR and ECG changes. It was hypothesised that due to the neuro- and cardio-protective effects of oestrogen, males would exhibit a more pronounced seizure response resulting in greater changes in ECG indices and cardiac morphology. The results of this study provide new insight into the ECG changes which occur during a seizure.

Figure 1.3: Proposed mechanism of SUDEP: enhanced autonomic activity leading to cardiac dysfunction. Oestrogen may have protective effects by reducing autonomic activity. Grey lines = normal communication between heart and autonomic nervous system, blue = sympathetic pathways, green = parasympathetic pathway, pink = oestrogen’s effects.
METHODS

2.1 Materials

All reagents were purchased from BDH (Palmerston North, New Zealand) and Sigma-Aldrich (Auckland, New Zealand). The prescription animal remedies, domitor, atropine, ketamine and Antisedan, were obtained from the University of Otago’s Drug Control Officer at the animal welfare office. Kainic Acid was purchased from Tocris (Bristol, UK) and dissolved in saline (0.9% NaCl).

2.2 Animals

Ten male and ten female Sprague-Dawley rats (300-350g, 2-3 month) were obtained from University of Otago Taieri-Hercus Breeding Station. The animals were housed in the Department of Pharmacology and Toxicology at the University of Otago on a 12-hour light/dark cycle at 22°C with food and water ad libitum. The animals were left to acclimatise for 5 days prior to surgery. Experiments were performed in accordance with the regulations of the University of Otago’s Committee on Ethics in the Care and Use of Laboratory Animals. Prior to surgery animals were housed three to five rats per cage according to gener. Both males and females were randomly allocated into either saline control animals or KA seizure-induced animals (n=5 per group).

2.3 Surgical Implantation of the Telemetric Transmitter

All animals underwent transmitter implantation surgery. The transmitters were sterilised in a 2% glutaraldehyde solution for 24 hr prior to surgery. The surgical instruments, plastic drapes and screws were sterilised with hibitane (95% ethanol/5% chlorhexidine acetate) for 30 min prior to surgery. Surgical swabs were autoclaved prior to surgery.

Animals were administered strepticin 250 IU and carprofen 5 mg/kg with 10 ml saline 30 min prior to surgery and once every 24 hours post-operatively for 3 days. Strepticin is an antibiotic used to prevent infection and carprofen was used for its analgesic and anti-inflammatory properties. Animals were anaesthetised with ketamine hydrochloride (75mg/kg, s.c.) and domitor (medetomidine hydrochloride 0.5 mg/kg, s.c.). A short acting dose of atropine (0.05 mg/kg, s.c.) was also used to decrease pulmonary mucus secretions and reduce bradycardia caused by domitor. The eye ointment, Tricin™, was applied throughout the surgery to prevent the cornea
drying out. Body temperature was maintained at 37°C throughout the surgery using a heating pad.

Transmitter implantation and electrode positioning was performed as previously described by Sawant et al. (2010). The abdomen, neck and scalp of the animal was shaved and sterilised with hibitane prior to surgery to reduce risk of infection. The animal’s claws were trimmed to prevent the rat interfering with its wound post-surgery. A surgical drape was placed over the animal exposing only incision sites. When pedal withdrawal reflex was abolished, a 3 cm incision was made in the skin overlying the lower abdomen in line with the xiphoid process. A two-channel digital transmitter (Telemetry Research, Auckland, New Zealand, 35 x 25 x 10mm, 16g; Figure 2.1) was inserted within a subcutaneous pocket.

![Figure 2.1: Two channel digital ECoG/ECG transmitter](image)

The reference ECG electrode was sutured to the dorsal surface of the xiphoid process using a non-dissolvable 4.0 silk suture (Ethicon). Using a trochar the recording ECG electrode was tunnelled subcutaneously to the rostral thorax. The trachea was exposed and the recording ECG electrode was pushed along the trachea into the anterior media stinum to position the electrode close to the right atrium. The lead was sutured to the tracheal muscle to keep it in the correct position during movement. The ECoG electrodes were tunnelled subcutaneously posterior to the foramen magnum. The abdomen was closed using a subcutaneous running suture (5-0 prolene suture, Ethicon) so the rat would not be able to open the wound. The neck was close using a standard surgical suture.

The animal was then positioned into a stereotaxic frame (David Kopf Instruments, USA), where an incision was made lengthwise along the skull and the overlying skin retracted. The top of the skull was cleaned with saline and the connective tissue pushed back. Bregma was used as the landmark for electrode placement.

Three holes were drilled into the skull at a depth of 1 mm. One for a stainless steel anchoring screw which was positioned 5 mm anterior to Bregma and 4 mm left of the midline. One for the reference electrode at 5 mm posterior of Bregma and 4 mm left of the midline. The third was for
the recording electrode at 5 mm posterior of Bregma and 4 mm right of the midline. The reference electrode was secured at a depth of 1mm from the skull surface using a stainless steel anchoring screw. The Kopf electrode holder was used to insert the recording electrode into the cortex at a depth of 2 mm from the top of the skull (Figure 2.2).

The electrodes were secured to the skull using cranioplastic cement and the electrode leads were also sutured to the muscle at the base of the skull to prevent displacement during seizures. The incision was then closed using a subcutaneous suture.

Antisedan™ (atipamezole hydrochloride, 0.5 mg/kg, s.c., an α2 adrenergic antagonist) was used to reverse the sedative effect of domitor. Warm saline (10 mL) was administered to reduce the dehydration caused by the surgery as domitor is associated with an increased urination rate. The animal was covered and left on the warming pad for an hour.

The animals were housed individually post surgery and left to recovery for 7 to 10 days before seizure induction. The rat was fed softened food pallets for a couple of days following operation. Animals and water intake were weighed and sutures checked daily to monitor recovery.
Seizure induction and Behavioural Observation

The behavioural study was performed in a custom-made Perspex™ observation chamber (Aburn Glass, Dunedin, New Zealand). The rat was left to acclimatise in the chamber for 30 min prior to study initiation. ECoG, ECG and behavioural data was recorded using Powerlab 2/25 signal conditioner and Chart 6 software (ADInstruments, Sydney). The ECG was sampled at 2000 Hz, with receivers set to 0.1 Hz high pass and 1000 Hz low pass filters.

Baseline recordings were taken over a 30 min period prior to saline control or seizure induction. Seizures were induced by a single injection of KA (10 mg/kg, s.c. from Saphire Bioscience, New South Wales, Australia). The control animals were treated with an equivalent volume of saline s.c (0.9% NaCl).

Following treatment the rat was immediately returned to the chamber for a 3 hour observation period. Behaviour was recorded every 15 sec with discrete changes in behavioural state additionally reported as they occurred. Behaviours were recorded using a 5-point scale (previously describe by (Ben-Air, 1985; Hesp et al., 2007; Sawant et al., 2010) Table 2:1). Level 0 = normal resting or exploratory behaviours, Level 1 = discomfort behaviours, Level 2 = stereotypical behaviours confined to the head and neck region, Level 3 = moderate seizure behaviours associated with stereotypical movements of the limbs or trunk, and Level 4 = severe generalised seizure behaviours leading to Level 5 clonic-tonic convulsions. ECoG, ECG and behavioural data was recorded for 3 hours post-treatment, with an hour observation period performed at 6 and 24 hours. Cumulative behavioural score during a specific time frame was calculated as the sum of the highest score taken every 15 sec.

<table>
<thead>
<tr>
<th>Level 0: Normal Behaviours</th>
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<tbody>
<tr>
<td>Sleeping</td>
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<tr>
<td>Walking</td>
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<td>Snuffing</td>
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<th>Level 1: Discomfort behaviours</th>
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<tr>
<td>Panting</td>
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<td>Abnormal resting</td>
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<th>Level 2: Mild seizures associated with stereotypical behaviours confined to the head and neck region</th>
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<tbody>
<tr>
<td>Freezing</td>
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<td>Head Shakes</td>
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<th>Level 3: Moderate seizure behaviours associated with stereotypical movements of the limbs or trunk</th>
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<tbody>
<tr>
<td>Forelimb Clonus</td>
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<td>Foot Biting</td>
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<th>Level 4: Severe generalised seizure behaviours leading</th>
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<tbody>
<tr>
<td>Rearing</td>
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<tr>
<td>Loss of Forelimb</td>
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<td>Balance</td>
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<th>Level 5: Clonic-tonic convulsions.</th>
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Table 2.1: Behavioural scores allocated to observed rat behaviours
2.5 Cardiac Perfusion

48 hours following KA or saline administration the animal was anaesthetised in a halothane bell jar. Anaesthesia was then maintained using a halothane nose cone. When pedal withdrawal reflex was abolished, the abdomen was cut using autopsy scissors from the left hind limb to the base of the sternum, below the diaphragm. The superior vena cava was then exposed and a 5 mL blood sample was taken using a heparinised 23G x ¼” needle. The heart was arrested in diastole by administering 1 mL of 20 mM KCl solution (20 mM KCl in 0.9% saline) into the vena cava bifurcation.

The heart was then excised and cannulated through the aorta so as perfuse the coronary arteries retrograde using a Langendorff perfusion rig with saline (4°C) to remove blood from the vasculature. Excess tissue such as lungs and adipose tissue were removed and the heart washed with saline. The heart was then perfused-fixed with 10% neutralised buffer formalin (NBF) solution and stored in NBF for 4 hours at 4°C. At the end of this period the heart was stored in 70% ethanol in preparation for paraffin embedding and sectioning. The blood sample was centrifuged at 3,000 rpm for 3 min and the plasma was stored at -80°C for future analysis.

2.6 Haematoxylin and Eosin staining

The cardiac ventricles were sliced into four 1.5 mm sections from the apex and stored in 70% ethanol until staining. Hearts were dehydrated and then paraffin wax embedded. Tissues were sliced with a microtome into 4 μm thick sections. Tissues were deparaffinised in xylene and rehydrated with absolute alcohol (1x 95% and 1x 70% ethanol). Slices were stained with Gills 2 Haematoxylin (Surgipath, Catalogue #01520) for 4 min. The sections were washed and stained in alcoholic eosin commercial for 1 min (Surgipath, Catalogue #01600). The slice was dehydrated in 70% ethanol and mounted in glass slides.

2.7 ECG and ECoG Analysis

Continuous ECG and ECoG data was recorded using Powerlab 2/25 signal transducer and Chart 6 software. ECoG was sampled at 2000 Hz, with receivers set to 0.1 Hz high pass and 1000 Hz low pass. ECoG was recorded during a 30 min acclimatisation period and for 3 hours following seizure induction. ECoG recordings were analysed in 10 min periods. Fast Fourier transformation was used to quantify the frequency bands of delta (1.25-4.5 Hz), theta (4.75-6.75 Hz), alpha (7.0-12.5 Hz), beta (12.75-35.0 Hz) and gamma (35.0-100 Hz). These were analysed by dividing them into half overlapping half-second epochs, using a weighted Cosine bell
window. Power Spectrum Density (PSD) was calculated as the sum of all epochs. Baseline was taken as the mean for each frequency band over the 30 min acclimatisation period.

ECG data was analysed using LabChart v.6 Pro ECG Analysis software (see appendix 5.1). HR, P duration, PR, QRS and QT intervals as well as P, R, S and T amplitudes were measured. Data was analysed every 5 min where a 1 min sample was analysed. This gave the mean changes over the 3 hour seizure observation period. Data was normalised to baseline taken as the mean of the 30 min baseline recording period and data was graphed as percentage of baseline. The QT interval was corrected using the Mitchell method, QTc = QT/(RR/100)\(^{1/2}\) (Mitchell, 1998).

Where high amplitude spiking occurred on the ECoG, 10 sec to 1 min periods of ictal activity were also analysed for ECG changes. 10 sec prior to seizure onset and 10 sec after was also analysed to examine pre and post ictal changes in HR and PR and QTc interval. HR over a 10 sec period was also analysed to determine if specific behavioural activities produced different HR responses.

### 2.8 Statistics

Statistical analysis was performed using Prism™ v.5. The behavioural data (cumulative score, WDS behaviours and Level 4 behaviours) were analysed using a non-parametric Mann-Whitney U test. ECG variables and HR changes over time were analysed using a using a 2-way repeated measures analysis of variance (AVOVA) to determine differences between groups over time. Variables were also compared to baseline using one-way repeated measures ANOVA. Statistical significance of ANOVA was determined with Bonferroni post hoc analysis. Linear regression was used to analyse the effect of HR on P wave amplitude. Statistical significance was determined as \(P<0.05\). Data was presented as mean ± standard error of the mean (SEM).
RESULTS

3.1 SEIZURE ACTIVITY

3.1.1 Behavioural Activity

Behaviours exhibited by saline control rats were the same as those taken during baseline recordings in all animals. Animals displayed exploring, grooming and resting behaviours. A mean cumulative score of 23.0 ± 3.1 for males and 17.9 ± 3.8 for females was recorded during the 30 min observation periods over the 24 hour behavioural study post saline.

Administration of KA (10 mg/kg, s.c.) resulted in a period of hypoactivity with seizure behaviours increasing in intensity 30 min post KA. Hypoactivity, such as resting in an abnormal posture and belly pressing generally occurred within 5 min (3.8 ± 0.4 min for males and 5.4 ± 0.9 min for females) of dosing with KA. This behaviour was accompanied by panting and squinting. Hypoactivity was followed by Level 2 behaviours such as freezing, mastication and head tremors. Onset of freezing was 17.8 ± 8.7 min post KA for males and 16.1 ± 1.2 min for females. Seizure activity continued to increase in intensity over the next 2 hours. Level 3 behaviours such as WDS and forelimb clonus were most commonly reported in males and myoclonic jerks, rearing and foaming was most prevalent in females.

Cumulative behavioural score increased over the first 2.5 hours post KA (Figure 3.1A). In males a maximum cumulative behavioural score of 367.8 ± 35.6 occurred during the 1-1.5 hour recording period (P<0.05 compared to baseline). Significant increases in cumulative behavioural score were also observed in females at 1 and 2 hour recording periods post KA (374.0 ± 22.9 and 396.3 ± 112.4, P<0.05 compared to baseline). The increase in seizure activity at 2 hours was significantly higher in females than in males (P<0.05), where cumulative behaviour scores dropped by 45% compared to scores recorded at the 1 hour recording period. In males WDS developed 41.5 ± 1.0 min post KA, with high intensity bursts which lasted more than a minute occurring at 66.2 ± 8.8 min. As shown in Figure 3.1B, WDS were significantly more prevalent in males, with 115.8 ± 19.5 behaviours recorded at 1-1.5 hours post KA (P<0.05 compared to baseline and females). This also correlated with the maximum behavioural score observed in males. Onset of WDS occurred later in females at 59.9 ± 4.7 min.

Level 4 behaviours were uncommon in males with an onset of 109 ± 13.9 min post KA (Figure 3.1C). In males, seizure activity decreased 2 hours after KA administration with normal exploratory and sleeping behaviours occurring at 127 ± 12.6 min, which were occasionally interrupted by Level 1 or Level 2 behaviours.
Figure 3.1: Effects of KA (10 mg/kg, s.c.) on mean behaviour recorded during a 30 min observation period in rats
A) Shows the sum of the maximum behavioural scores recorded every 15 seconds over a 30 min observation period. B) Number of wet dog shake (WDS) behaviours observed. C) Number of Level 4 seizure behaviours. Values represented as the mean ± SEM, n=4-5 per group. * = P<0.05 compared to baseline in males, # = P<0.05 compared to baseline in females, ° = P<0.05 compared to females.
In females, KA administration resulted in significant increases in Level 4 and Level 5 behaviours compared to KA males. Increased Level 4 behaviours (35.0 ± 23.0) occurred over the 2-2.5 hours post KA ($P<0.05$ compared to baseline and KA males, Figure 3.1C). No Level 5 behaviours were observed in males. However tonic-clonic seizures occurred in 4 of the 5 KA female rats. In females, a number of Level 5 behaviours (7.6 ± 4.9) occurred during the 2-2.5 hour ($P<0.05$ compared to baseline and KA males). Female #1 had the highest rate of Level 5 behaviours with 26 events during the 2-2.5 hour recording period and a single episode also observed 6 hours post KA.

**Figure 3.2:** Shows examples of ECoG traces recorded 60-90 min following KA (10 mg/kg, s.c.) administration in male rats. First panel shows a 90 sec recording period of behaviour, second panel shows an expanded section of the area represented within the box. Each trace represents ECoG activity recorded during: A) Sniffing. B) Freezing. C) Mastication. D) Head tremors. E) Salivation. F) Myoclonical Jerks. G) Tonic-clonic convulsions.
Figure 3.2 shows examples of ECoG traces demonstrating specific seizure patterns which occurred 60-90 min post KA. Seizure behaviour was associated with an increase in frequency and amplitude of spikes. Exploratory behaviours such as sniffing were coupled to low frequency, high amplitude spiking. Freezing behaviours resulted in low amplitude spiking with peak frequencies of 2.9 and 37.1 Hz following FFT analysis. Myoclonic jerks and tonic-clonic convulsions were associated with high amplitude low frequency delta activity.

### 3.1.2 Power Spectrum Density

![Graph of Power Spectrum Density](image)

**Figure 3.3**: Effect of KA (10 mg/kg, s.c.) on Power Spectrum Density (PSD) from ECoG recording in rats. PSD was determined by performing Fast Fourier transformation of ECoG seizure activity. Graphs represent 10 min recordings of each frequency band: delta (1.25-4.5 Hz), theta (4.75-6.75 Hz), alpha (7.0-12.5 Hz), beta (12.75-35.0 Hz) and gamma (35.0-100 Hz). Values normalised to 30 min baseline recording and presented as mean ± SEM. n=3 for males, n=4 for females. * = P<0.05 compared to male. # P<0.05 compared to baseline.
Power Spectrum Density (PSD) shows that there were significant differences between males and females during theta, delta and alpha frequencies. Power increased over time and this correlated to an increase in seizure severity. During the 3 hour observation period, females showed significant increases in delta, theta and alpha power during the 10 min recording period at 120, 130, 160 and 170 min (P<0.05 compared to baseline and KA males, Figure 3.3). These increases in PSD occurred during Level 4 and Level 5 seizures in females. There was also a significant increase in PSD for alpha and beta frequency bands during the 50-60 min recording period (P<0.05 compared to males and baseline). The theta frequency band was associated with the greatest increases in power, with a maximum increase of 34052 ± 11982% at 140 min. Seizure activity in males did not produce any significant increases in PSD over these frequency bands. No significant difference in PSD was observed between males and females in the gamma frequency band, although there was a significant increase in PSD at 110 min for males and 100 min for females when compared to baseline (P<0.05).

3.1.3 Seizure Level and Heart Rate

Animals exhibited Level 1 and 2 seizure behaviours in the saline treated animals and during baseline in KA treated animals. These included Level 1 behaviours such as blinking, Level 2 behaviours such as mastication and WDS. Level 4 or Level 5 behaviours were not observed at anytime in the saline dosed animals or during baseline in the KA animals. A mean if 153.0 ± 31.9 behaviours were analysed per level to determine the HR changes.

KA administration (10 mg/kg) in males and females resulted in significant decreases in HR during Level 0 or Level 1 activity (P<0.05 compared to saline and baseline; Figure 3.4). This hypoactive period was observed during the first 30 min and was associated with rapid decreases in HR (Figure 3.4). In males a reduction of 48.7 ± 11 bpm and 130.5 ± 8.4 bpm occurred during Level 0 and Level 1 behaviours, respectively. In females this decrease was even greater with a decline in HR of 104 ± 15.8 bpm and 210.7 ± 19.8 bpm, respectively (P< 0.05 compared to baseline and KA males). Females also had a significant reduction in HR of 90.1 ± 19.2 bpm while exhibiting Level 2 behaviours (P<0.05 compared to KA males, Figure 3.4C). Non-significant increases in HR of 40-60 bpm were observed during Level 3 and Level 4 behaviours in both males and females. Level 5 seizure activity in females did not result in HR changes (398.0 ± 10.9 bpm).
Figure 3.4: Mean heart rate (bpm) recorded during 10 sec of behavioural activity in male and female rats. Animals received saline or KA (10 mg/kg) following a 30 min baseline recording period. A) Compares saline to KA males. B) Compares saline to KA females. C) Compares KA males and KA females. Values represent the mean ± SEM, n=4-5 per group. *= P<0.05 compared to baseline in males, #= P<0.05 compared to baseline in females, °= P<0.05 compared to females.
Figure 3.5: The gray trace represents a compressed ECoG trace and the black line shows the mean heart rate recorded in synchrony over a 210 min observation period in representative individual male (A and B) and female (C and D) Sprague-Dawley rats. The first 30 min represent baseline activity prior to KA (10 mg/kg) administration (Arrow). Heart rate was taken as the mean of single 1 min recordings taken every 5 min.

Figure 3.5 shows a compressed ECoG trace with HR recorded every 5 min over the 3 hour observation period. During the 30 min baseline recording period HR did not significantly change. Prior to KA administration the ECoG generally remained constant with occasional spiking due to movement. Immediately following KA administration, bradycardia ensued in both sexes with infrequent spiking in ECoG traces. The amplitude of ECoG spiking increased with high intensity seizure activity. Robust high amplitude spiking occurred 60 min post KA which was associated with periodic increases in HR.
3.2 HEART RATE

Baseline HR for male and female Sprague-Dawley (300-350 g) rats in the saline control group was 425.5 ± 10.1 and 423.3 ± 16.5 bpm, respectively. There was no difference in baseline HR in the KA groups and no gender differences were present (P>0.05).

Bradycardia

Administration of KA (10 mg/kg, s.c.) in males resulted in a significant decrease in HR by 20% within 10 min which was maintained for 30 min (P<0.05 compared to baseline, Figure 3.6) before returning to baseline at 75 min. A mean minimum HR of 71.2 ± 7.3% occurred 20 min following KA injection. The decrease in HR was non-significant compared to saline control animals.

KA administration in female Sprague-Dawley rats resulted in a rapid significant decrease in mean HR by 23.4 ± 1.0 % within 5 min (P<0.05 compared to saline control females, Figure 3.6). HR continued to decrease for 20 min, until a minimum HR of 55.7 ± 2.4 % occurred at 50 min.

Figure 3.6: Effect of KA (10 mg/kg, s.c.) on mean heart rate in rats over a 210 min period. (arrow). Control animals were administered saline at 30 min. Values represent mean normalised HR (%) for a 1 min recording period every 5 min ± SEM. Baseline heart rate taken as heart rate at 30 min. n=4-5 per group. *= P< 0.05 KA males compared to saline males. #= P< 0.05 KA females compared to saline females
HR remained significantly decreased for 45 min post KA before starting to return to baseline ($P<0.05$ compared to baseline and saline control females).

Male #2 showed the greatest decreases in HR compared to baseline. A decrease in HR of 20.1% occurred within 5 min with a maximum decrease of 55.0% recorded 15 min post KA ($P<0.05$ compared to baseline). In this male a decrease in HR of greater than 30% was maintained for 50 min following KA administration. Male #2 took longer to return to baseline (98.6% at 95 min) and demonstrated non-significant increases in HR 90 min post KA. The lowest recorded mean HR during a 1 min period occurred in Female #1 who had a HR of 154.7 bpm at 10 min post KA, while the lowest HR in males was 40 bpm higher (195.3 bpm in Male #2, Table 3.1).

### 3.2.3 Tachycardia

Continued monitoring showed that at 100 min HR subsequently increased in KA dosed male rats by 25.2 ± 7.1% compared to baseline. This significant increase in HR by greater than 20% lasted for 50 min ($P<0.05$ compared to baseline and saline dosed males) with maximum HR reaching 127.2 ± 5.0% at 115 min post KA (Figure 3.6).

Mean normalised HR for females increased by 22.3 ± 6.4 at 95 min ($P<0.05$ compared to baseline). HR remained significantly elevated until 175 min ($P<0.05$ compared to baseline and saline dosed females). A maximum HR of 131.4 ± 9.5% occurred 75 min post KA. HR returned to baseline 3 hours following KA administration. Male #1 and Male #4 showed the greatest increases in HR with an increase of 41% recorded above baseline recorded. Male #4 had the highest recorded HR of 510.1 bpm (Table 3.1). Overall there were no significant differences between individual animals during the 210 min recording period. No significant difference in HR occurred between male and female groups.
3.2.4 Heart Rate 24 hours Post KA

KA administration in male rats resulted in a significant increase in mean normalised HR during the 1-2 hour and 2-3 hour recording period by 20.6 ± 4.5% and 11.6 ± 4.4%, respectively ($P<0.05$ compared to baseline, Figure 3.7). KA administration in females resulted in a significant decrease in HR by 28.0 ± 3.8% during 0-1 hour following KA administration. An increase of 22.9 ± 10.0% and 17.7 ± 9.7% occurred at 1-2 hours and 2-3 hours, respectively ($P<0.05$ compared to baseline). Mean normalised HR for male and female KA groups returned to baseline levels at the 6 and 24 hour recording periods (ranged from 89.3 ± 3.6% to 101.3 ± 7.6%). There were no significant differences in mean HR between saline control groups or between KA male and female groups.

**Figure 3.7:** Effects of KA (10 mg/kg, s.c) on mean heart rate in rats over 24 hours. Baseline heart rate was recorded as the mean heart rate over a 30 min recording period prior to KA administration. Values represent mean normalised HR (%) for a 1 hour recording period ± SEM. n= 4 for males, n= 5 for females. *$= P<0.05$ compared to baseline in males, #$= P<0.05$ compared to baseline in females,
| Male #1 | Level 4 | Forelimb clonic tonic | 1544 | 497.3 | 278.2 | 95.2 | 123.4 | No P wave |
| Male #2 | Level 4 | Forelimb clonic tonic | 928  | 505.4 | 195.3 | 107.4 | 105.2 | No P wave, Long PR interval, Periods with no P wave, Presence of a P wave during a skipped beat, atrial depolarisation without ventricular contraction, Skipped beats, Variations in R and S amplitudes |
| Male #3 | Level 3 | WDS | 1419 | 496.9 | 292.4 | 98.7 | 116.9 | Slight decrease in P wave amplitude with decreased HR, Variations in R and S amplitudes |
| Male #4 | Level 4 | Forelimb clonic tonic | 1315 | 510.1 | 283.5 | 101.0 | 118.9 | Reduced P wave and R wave amplitude during bradycardia, Oedema with loss of structural integrity, Myocyte vacuolisation |
| Female #1 | Level 5 | Clonic tonic convulsions | 2444 | 498.2 | 154.7 | 127.4 | 115.6 | Skipped beats, Irregular heart beats, No P wave |
| Female #2 | Level 4 | Forelimb clonic tonic | 1091 | 545.1 | 258.9 | 104.9 | 98.2 | No or inverted P waves, Long PR interval |
| Female #3 | Level 5 | Clonic tonic convulsions | 1695 | 490.6 | 211.0 | 104.6 | 109.8 | No or inverted P waves, Long PR interval |
| Female #4 | Level 5 | Clonic tonic convulsions | 1619 | 477.9 | 215.6 | 106.4 | 106.0 | Reduced P waves, Long PR interval, Skipped beats |
| Female #5 | Level 5 | Clonic tonic convulsions | 1461 | 510.3 | 187.3 | 150.1 | 112.8 | Long PR interval |

**Table 3.1:** Summary of behavioural, ECG and histology results in rats over 3 hours following KA (10 mg/kg). HR = Heart rate, RBC = Red blood cells, WDS = Wet dog shakes
3.3 ECG CHANGES

3.3.1 PR Interval

The mean baseline PR interval for KA dosed males and females was 46.5 ± 1 msec and 41.4 ± 5 msec, respectively. No significant difference in baseline PR interval was found between all groups. No change in PR interval occurred following KA administration in the males (Figure 3.8). However, PR interval increased by 20% at 45 min following KA administration in the female group. This increase was significant at 55, 85, 135 and 140 min ($P<0.05$ compared to saline control, Figure 3.8) with a maximum increase of $35.2 ± 18.2\%$ occurring 55 min post KA in the females.

![Figure 3.8](image_url)

*Figure 3.8: Effects of KA administration on mean PR interval in rats over a 210 min observational period. Animals received saline or KA (10 mg/kg) following a 30 min baseline recording period (arrow). Values represent mean normalised PR interval (%) for a 1 min recording period every 5 min ± SEM. Baseline PR interval taken as PR interval at 30 min. n= 4-5 per group. # = $P< 0.05$ compared to saline females*
3.3.2 P wave Duration

Baseline P wave duration in male and female Sprague-Daley rats was $17.9 \pm 0.4$ msec and $14.3 \pm 1$ msec, respectively. No significant differences in the mean normalised P wave duration was seen between baseline and saline control animals.

In the male KA group, there was a significant decrease in the P wave duration by $29.4 \pm 11.4\%$ at 40 min (Figure 3.9). The P duration continued to significantly decrease by greater than 30% at 45 and 50 min ($P<0.05$ compared to baseline). This decrease in P wave duration occurred during the bradycardic period and was significantly different compared to KA administered females where no significant changes in the P wave duration occurred ($P>0.05$, data not shown).

![Figure 3.9: Effect of KA on mean P wave duration in male rats over a 210 min observational period. Animals received saline or KA (10 mg/kg) following a 30 min baseline recording period (arrow). Values represent mean normalised P Duration (%) for a 1 min recording period every 5 min ± SEM. Baseline P wave duration taken as P duration at 30 min. n=4 per group. *= $P<0.05$ compared to baseline, #= $P<0.05$ compared to saline males]
3.5.3 QTc Interval

The recorded QT interval was corrected using the Mitchell method, \( QTc = \frac{QT}{(RR/100)^{1/2}} \) (Mitchell, 1998). As the HR increases, it produces a proportional reduction in the RR interval, resulting in a reduced QT interval. The converse was also true as HR decreased. Consequently the QTc was employed to correct for this and to determine if there was abnormal shortening or lengthening in the QT interval.

Baseline QTc Interval in male and female Sprague-Daley rats was 41.9 ± 4 msec and 52.7 ± 6 sec, respectively. No significant difference in mean normalised QTc intervals was seen across all groups.

KA administration in females resulted in a significant decrease in the QTc interval by 29.9 ± 5.5% and 28.7 ± 4.4% at 40 and 45 min, respectively (\( P<0.05 \), Figure 3.10). In the male KA group, the QTc interval significantly increased by 34.8 ± 4.7% at 100 min, with increases of greater than 20% occurred at 105, 115, 120 and 150 min (\( P<0.05 \) compared to baseline). Although KA administration resulted in a 20% increase over baseline in the QTc interval at 95 and 100 min in female rats, this effect was not significant. Although there were significant changes in the QTc interval in the male and female KA treated groups, these were not significantly different between the sexes.

![Figure 3.10](image-url): Effect of KA on mean QTc Interval in rats over a 210 min observational period. Animals received saline or KA (10 mg/kg) following a 30 min baseline recording period (arrow). Values represent mean normalised QTc Interval (%) for a 1 min recording period every 5 min ± SEM. Baseline QTc interval taken as QTc interval at 30 min. \( n=4-5 \) per group. \# = \( P < 0.05 \) compared to saline males. \# = \( P < 0.05 \) compared to saline females.
The mean P wave amplitude for baseline in the male KA group was 0.09177 ± 0.0301 mV. This was non-significantly different compared to female or saline control animals. As shown in Figure 3.11, there was high variability in the P wave amplitude during the recording period. No significant changes in P wave amplitude were observed following KA administration in males when compared to baseline or saline control rats (P>0.05, data not shown). KA administration in females resulted in a significant decrease in P amplitude within 5 min and lasting for 40 min (Figure 3.11). The P amplitude decreased to 28% at 45 and 50 min (P<0.05 compared to baseline and saline). There was a significant difference between male and female P wave amplitudes during the bradycardic period (30-75 min).

The change in P wave amplitude correlated with the changes in HR in the KA groups (Figure 3.12). There was a weak relationship between P amplitude and HR in male and female saline control animals, with an R² of 0.0414 and 0.6287, respectively (Figure 3.12A). However KA administration resulted in a strong correlation between P amplitude and HR. KA administration in males had a linear regression of y = 2.0205x - 96.187 with a strong R² of 0.9094 (Figure 3.12B). A similar relationship also occurred in the female KA group where KA administration resulted in a linear regression of y = 1.4382x - 48.82 with an R² of 0.9176.

Figure 3.11: Effect of KA on mean P wave amplitude in female rats over a 210 min observational period. Animals received saline or KA (10 mg/kg) following a 30 min baseline recording period (arrow). Values represent mean normalised P wave amplitude (%) for a 1 min recording period every 5 min ± SEM. Baseline P wave amplitude taken as P wave amplitude at 30 min. n=5 per group. #= P< 0.05 compared to baseline.


3.3.5 Other ECG variables

KA administration resulted in no differences in the QRS interval, R amplitude or T amplitude compared baseline or saline control rats. No sex differences were observed either. No change in the S amplitude occurred in the male KA group ($P > 0.05$, data not shown). However, KA administration in females resulted in a significant increase in the S Amplitude by 115.4 ± 85.8% and 100.8 ± 87.0% at 60 and 65 min, respectively ($P < 0.05$ compared to baseline and KA males, Figure 3.13). A 96.9 ± 44.7% increase in S wave amplitude also occurred at 205 min ($P < 0.05$ compared to baseline and saline control females).

Figure 3.12: Mean normalised P wave amplitude in male (■) and female (♦) rats compared to mean normalised heart rate over a 210 min observation period in A) saline control animals and B) KA (10 mg/kg) dosed animals. Values represent mean P wave amplitude and heart rate normalised (%) against baseline for a 1 min recording period every 5 min. Data normalised to baseline measurement at 30 min. n= 4 -5 per group

Figure 3.13: Effect of KA on mean S wave amplitude in female rats over a 210 min observational period. Animals received saline or KA (10 mg/kg) following a 30 min baseline recording period (arrow). Values represent mean normalised S Amplitude (%) for a 1 min recording period every 5 min ± SEM. Baseline P Amplitude taken as S wave amplitude at 30 min. n=5 per group. *= $P < 0.05$ compared to baseline and saline females
3.3.6 Specific ECG abnormalities

The main ECG abnormalities which occurred following KA induced seizures were skipped beats and changes in the P wave. Skipped beats occurred in three rats (one male and two females) during the bradycardic period (Figure 3.14D and E). During bradycardia there were changes in the P wave in all animals. Persistent decreases in P wave amplitude frequently occurred during the first 30-60 min post KA. Abnormal atrial depolarisation was the most common ECG abnormality recorded (Table 3.1). Female #2 experienced inverted P waves. Male #2 presented with small amplitude P waves occurring during skipped beats (Figure 3.14D). This suggests that atrial depolarisation occurred without ventricle depolarisation.

![Figure 3.14: Examples of ECG abnormalities which occur 10-20 min post KA (10 mg/kg, s.c.) in Sprague-Dawley rats (300-350g). Scale= 0.1 sec x 0.2 mV. A) Male #1: Baseline. B) Male #1: Bradycardia. C) Male #2: Baseline. D) Male #2: Skipped beats. E) Female #1: Skipped beats. F) Female #1: Irregular heart beats. G) Female #2: Inverted P wave. H) Female #5: Tachycardia at 90 min post KA]
3.3.7 Specific ECG Abnormalities during Ictal Activity

Ictal events were also analysed to see if seizure activity caused specific changes in HR, PR interval or QTc interval. 10 sec of pre-ictal and post-ictal data was analysed as clinical data has demonstrated that the HR change can precede the seizure onset. There were no significant differences in pre-ictal, ictal or post-ictal HR, PR interval or QTc interval when the data was averaged between animals (P>0.05, data not shown). However it was noticed that there were outliers in the data so changes of greater than 10% of baseline were recorded per animal.

In males a HR increase was 2.8-fold more common than in females, occurring in 10% of seizure events. Changes in PR interval were more common in males than females occurring in 13.7% of seizures. A decrease in QTc interval of greater than 10% was reported in 56% of ictal events, although half of these occurred in Male #1. Decreases in QTc interval occurred in 7.2% of seizure events which was lower than changes observed in females.

In females, a decrease in HR greater 10% occurred in 9.3% of seizure events. The PR interval was altered in 9.2% of seizures, with an increase if greater than 10% in 4.2% of seizure events. Changes of greater than 10% in the QTc interval were observed in 28.0% of seizure events. In 16.9% of seizures the QTc interval increased by greater than 10%.

Seizure activity in Male #1 showed large variability in the QT interval. Changes of greater than 10% ranged from -19.8 to 9.01 ms. Most commonly observed were decreases in the QT interval which occurred in 76 of 93 seizure events. Male #4 showed the greatest changes in HR. Seizure activity at 150 min resulted in a massive increase in HR of 108.6 bpm where HR rose to 526.1 bpm post-ictally.

Female #1 and #5 showed the most pronounced and frequent ictal changes. Female #1 had three ictal events where HR dramatically increased between the pre and post ictal period. These increases ranged from 143.6 to 212.3 bpm and occurred 56-97 min post KA. In this animal there were also decreases in the PR interval of greater than 10% observed prior to 50% of ictal events. Female #5 presented with changes in the QTc interval in 27 of 38 events, with dramatic decreases occurring in 38% of ictal events.
3.6 HISTOLOGY

Haemotoxylin and eosin stain (H & E) staining of cellular architecture is used to determine if cellular damage has occurred such as necrosis, fibre tearing, haemorrhage or macrophage infiltration. Haemotoxylin stains the nucleus blue while eosin stains cytoplasm, collagen and muscle fibres pink. Infiltration of inflammatory cells, haemorrhage and hypercontracture band necrosis were observed 48 hours post KA induced seizures (Figure 3.15). The most commonly observed cardiac pathology was oedema which occurred in hearts from all KA animals. Myocyte vacuolisation was also observed in several hearts and this is a marker of early reversible ischaemic damage (Tsang et al., 2008a). Similar damage was observed as microfoci throughout the subendocardium and papillary muscles of the left ventricle in male and female KA treated animals. Female #1 exhibited more pronounced seizure activity than all other animals. H & E staining of this animal’s heart revealed oedema and infiltration of inflammatory cells similar to those observed in other animals. Red blood cell (RBC) extravasation and infiltration within the ventricular muscle fibres seen as evidence of intramyocardial haemorrhage was also present. Female #3 also showed histological evidence of RBC infiltration (Figure 3.15D). This animal had the second highest rate of tonic-clonic convulsions with 5 episodes recorded over the 3 hours post KA.
Figure 3.15: Haematoxylin and eosin staining of the left ventricular myocardium 48 hours following saline or KA (10 mg/kg, s.c.) administration in rats. A) Saline control male: normal histology. B) Male #4: reversible cardiomyopathy evidenced by myocyte vacuolisation. C) Female #1: infiltration of inflammatory cells. D) Female #3: red blood cells extravasation and inflammatory cells. E) Female #3: myocyte vacuolisation and red blood cells. F) Female #5: hypercontracture band necrosis and inflammatory cells.
DISCUSSION

4.1 SEIZURE ACTIVITY

KA induced seizures were used as an animal model of TLE. KA causes hyperexcitability throughout the brain by activating AMPA and kainate receptors. The hippocampus is more susceptible to KA’s effect than other brain regions, due to its high concentration of KA binding sites and Ca$^{2+}$ permeable AMPA receptors (Ben-Air, 1985; Lothman and Collins, 1981; Wisden and Seeburg, 1993). Binding of KA to AMPA and kainate receptors can produce excitotoxicity due to hyperdepolarisation of the neuron and activation of NMDA receptors and VGCC causing further neuronal damage.

In the present study, seizure activity induced by KA resulted in a period of hypoactivity followed by freezing behaviours. Similar to previous studies, animals exhibited staring behaviours and decreased responsiveness to stimuli before developing limbic seizures (Lothman and Collins, 1981; Chen et al., 2002). The severity of seizure activity increased for at least 2 hours post KA, progressing to generalised seizure activity with increased locomotion and rearing behaviours. WDS occurred earlier and were 3.5-fold more prevalent in males than females. In general, females experienced higher KA induced seizure behaviours than males. A high incidence of Level 4 behaviours such as loss of balance, rearing and myoclonic jerks were observed in all females and these progressed to clonic-tonic convulsions in four animals. No Level 5 behaviours were observed in males. These results suggest that oestrogen has a pro-convulsant effect as the severity and duration of seizure behaviours were more intense in females.

The severity of the seizures increased over time before returning to baseline levels. Systemic administration of KA may result in a progressive increase in neuronal KA levels as more crosses the blood brain barrier. KA induced seizures increases the permeability of the blood brain barrier which may act to facilitate this effect (Zucker et al., 1983). The increase in seizure severity may be due to elevated KA levels resulting in non-specific binding or spreading of electrical activity from the hippocampus to cortex producing the generalised seizure activity observed 60 min post KA.

The results from this experiment were similar to those previously reported by Goulton et al. (2010). ECoG data was used to examine the effect of a high dose of KA (10 mg/kg, s.c.) in male Sprague-Dawley rats. KA produced seizures which steadily increased during a 2 hour observation period. Similar to the present study, WDS were most commonly observed occurring 150 times during the 2 hours post KA. Level 4 behaviours were less common with an
average of 35 events recorded. KA administration resulted in several minutes of hyperactivity and exploratory behaviours followed by a short period (15 min) of hypoactivity. Within 20 min head tremours, mastication, freezing and WDS occurred with increasing frequency.

Experiments using domoic acid also support the behavioural activity observed in this experiment. Domoic acid, like KA, is a potent agonist for AMPA and kainate receptors. It induces behaviours similar to KA, such as WDS, freezing, tremors and eventually tonic-clonic convulsions in rats. Domoic acid initially affects limbic structures such as the hippocampus and progresses to generalised epileptiform activity. At high doses or in susceptible animals it can cause death from SE (Hesp et al., 2007; Sawant et al.,2010). A systemic low dose of domoic acid (0.5 mg/kg, i.p.) in male rats produces Level 1 and Level 2 seizure behaviours, whereas a higher dose of 2 mg/kg produces progressively stronger and more frequent seizure activity (Hesp et al., 2007). At 2 mg/kg domoic acid produced Level 4 seizures in all animals. The onset to WDS was 61.5 min longer than observed in the present experiment. This may be due to domoic acid having a different affinity for AMPA and kainate receptor subunits in different brain regions. An intra-hippocampal dose of domoic acid (100 pmol) also produced comparable results. Head tremors, mastication, freezing and WDS occurred within 5 to 20 min. Enhanced behavioural activity was associated with high frequency/high amplitude spiking. Level 4 behaviours occurred approximately 40 min post domoic acid and these were associated with robust increases in ECoG power (Sawant et al., 2010). The administration of a low dose of intrahippocampal domoic acid produced the same behavioural trend observed in the present study. This suggests that the increase in severity of seizure behaviour following systemic KA is linked to spreading of the electrical activity rather than elevated levels of KA within the CNS.

Other experiments involving specific brain regions have also observed an increase in locomotor activity during seizure activity. Bilateral injections of KA (0.1-1.0 pmol) into the dorsomedial hypothalamus (DMH) of male rats produced dose-dependent increases in HR, which correlated to an increase in locomotor activity such as running, rearing, WDS and grooming. Da Silva et al. (2006) found that micro-bolus injections of NMDA (3, 6 and 12 pmol) into the periaqueductal gray area produced an increase in HR and BP which was accompanied by elevated locomotor activity. Substituting NMDA with KA (1, 6, 12 and 50 pmol) however, produced small non-significant increases in HR, BP and locomotion, suggesting receptor specific effects.

In the present study, females showed greater increases in PSD than males for delta (1.25-4.5 Hz), theta (4.75-6.75 Hz) and alpha (7-12.5 Hz) frequency bands. Activation of these lower frequencies is associated with neurological diseases such as TLE (Zaveri, 2001), which is
consistent with KA induced seizures being an animal model of TLE. The increases in ECoG power were associated with enhanced seizure activity. The greatest increases in PSD occurred 2 hours post KA when animals exhibited Level 4 and Level 5 seizures.

The theta band is associated with hippocampal activity (Michel et al., 1992). Significant increases in theta power were observed following KA administration in females which is consistent with KA administration initiating seizures in the hippocampus. Oestrogen has been reported to potentiate the effect of KA currents in the hippocampus by increasing the density of NMDA receptors in the CA1 region (Gu et al., 1999). Therefore the presence of oestrogen in females enhances excitatory transmission in the hippocampus, which results in increased spiking on the ECoG.

In the male KA group, the increases in PSD were similar to the data reported by Goulton et al. (2010) and Sawant et al. (2010). Goulton et al. (2010) observed changes in ECoG signal intensity as early as 10-15 min, with peak changes from 90-120 min post KA. Level 3 and Level 4 behaviours occurred 40 min post KA and were associated with robust increases in ECoG power similar to those observed in the present study.

The data in this experiment suggests that oestrogen has pro-convulsant effects. Females exhibited higher seizure behaviours which remained elevated during the 3 hour observation period, whereas behaviour tended to return to baseline after 2 hours in males. Females also had a shorter latency to the onset of Level 4 behaviours than males. This contrasts with a study by Veliskova et al. (2000) who found that oestrogen replacement therapy in ovariectomised rats delayed the onset of clonic seizures. Animals were hormone replaced with β-oestradiol (2 µg) 48 and 24 hours prior to KA induced (16 mg/kg, i.p.) seizure activity. Oestrogen treatment prior to KA significantly delayed the onset of KA induced clonic seizures by 15.7 min. Oestrogen also protected against KA induced seizure-related mortality with all animals surviving compared to five of nine in the control group. However oestrogen administered post KA had no protective effects.
4.2 HEART RATE AND SEIZURE ACTIVITY

4.2.1 Bradycardia

In the present study, administration of KA resulted in a decrease in HR in males and females. In males there was a decrease in HR by ~30% within 30 min post KA. The bradycardic effect was more prominent in females where KA administration resulted in a rapid and pronounced decrease in HR by 20% within 5 min, reaching a mean minimum HR of 56% at 20 min. Analysis of pre- and post- ictal HR showed that HR deceased by greater than 10% in 11.5% of seizures. The decrease in HR has been proposed to be caused by changes in autonomic function induced by seizure activity. Ferrari et al. (2008), examined the effect of systemic KA in male rats. The animals underwent a bilateral adrenalectomy or sham operation 48 hours prior to a single injection of KA (12 mg/kg, i.p.). Systemic KA decreased BP by 35% and HR by 22% in the sham operated animals. This bradycardic and depressor response occurred 15 min following injection and lasted for 8 hours. In animals which underwent an adrenalectomy, the effect of KA on HR and BP was not diminished. This suggests that the effect produced by systemic KA is independent of the sympathetic system and most likely due to enhanced parasympathetic nervous activity.

Ictal bradycardia is an uncommon phenomenon reported in clinical studies, occurring in fewer than 2% of seizures (Nei et al. 2000; Leutmezer et al., 2003; Devinsky, 2004). Bradycardia appears to have a higher incidence in seizures of temporal or frontal lobe origin and is more frequent in males and patients with left-sided seizure foci (Devinsky, 2004). Ictal bradycardia tends to start 10 to 30 sec after the EEG onset of the seizure (Sevcencu and Struijk, 2010).

In a study in the United Kingdom twenty epileptic patients with refractory partial seizures were implanted with a loop ECG recorder for 24 months (Rugg-Gunn et al., 2004). Ictal bradycardia occurred in 35% of patients (2.1% of seizures). Four patients (21%) required a permanent pacemaker due to bradycardia or asystole and all four patients were men. Three of these four patients had seizures arising from the left hemisphere and three had TLE. Another three patients had severe bradycardia with HR of less than 30 bpm lasting for 10 to 15 sec.

It is possible that the occurrence of ictal bradycardia may be underestimated as it can go unnoticed. Zijlmans et al. (2002) retrospectively analysed 281 seizures from 81 patients with epilepsy who had prolonged EEG and ECG recordings. Increase in HR was most common, occurring in 93% of seizures but in 7% of seizures (15% of patients) HR decreased by at least 10 bpm. A Singapore based study in 37 non-Caucasian patients with partial seizures also
reported a high incidence of sinus bradycardia occurring in 16% of seizures (Wilder-Smith and Lim, 2001).

Bradycardia leading to asystole may be implicated in the pathology of SUDEP. Asystole has been reported to occur in 0.27 to 1.2% of patients with epilepsy (Scott and Fish, 2000; Zijlmans et al., 2002; Rocamora et al., 2003; Schuele et al., 2007). Asystole is more common in seizures of temporal lobe origin and has not been recorded in patients with generalised seizures. The occurrence of asystole has been reported as high as 2.3% of seizures and 21% of patients (Nei et al., 2000; Rugg-Gunn et al., 2004). Rocamora et al. (2003) reported ictal asystole in 5 of 1244 epileptic patients. The asystole lasted 4-60 sec and a pacemaker was required in all patients. Four of the patients had left sided seizure origin and one was bifocal.

Although ictal bradycardia is rare and transient, it cannot be ruled out as a contributor to SUDEP. Bradycardia can progress to a potentially fatal asystole (Sevcencu and Struijk, 2010). If the asystole lasts more than 10 sec it can cause severe hypoxemia which could lead to a fatal decrease in cerebral oxygen supply and sudden death (Surges et al., 2009). Prophylactic implantation of a permanent pacemaker may decrease mortality in at-risk patients.

4.2.2 Tachycardia

In the present experiment, seizure activity induced by systemic KA did not result in a high incidence of ictal tachycardia which is commonly reported in clinical based literature. KA administration in males resulted in an increase in HR by 25% at 70 min post KA which lasted for 50 min. This effect was more prolonged in females, with HR increasing at 65 min and remaining significantly elevated for 2 hours. Pre and post ictal analysis of HR showed that in 21.2% of seizure events there was an increase in HR of greater than 10%. The delay in the onset of tachycardia may be a reflex response to the bradycardia induced by KA administration or it could be due to the spread of seizure activity to other brain regions.

Tachycardia most likely occurs due to enhanced sympathetic stimulation. Hotta et al. (2009) examined the effect of KA (10 mg/kg, i.p.) induced limbic seizures in urethane-anesthetised male rats. Cardiac sympathetic nerve activity showed a significant 2-fold increase during seizure activity. KA administration resulted in a significant 15% increase in HR from 348 bpm to 408 bpm. This is similar to the tachycardia observed in the males in the present study. Hotta et al. (2009) used the combination of urethane and KA to produces limbic cortical seizures without motor convulsions. This allowed recordings to be made without movement artefacts.
Tachycardia is the most commonly reported autonomic symptom reported during seizures. It is reported to occur in up to 100% of seizures (Opherk et al., 2002; Surges et al., 2009; Jansen and Lagae, 2010) and can precede the EEG onset of the seizure by 8-19 sec (Zijlmans et al., 2002; Devinsky, 2004; Sevcencu and Struijk, 2010). Patients with epilepsy are also associated with a mean higher HR than the general population. Interictal HR was recorded in 14 male and 11 female patients with frontal lobe epilepsy (Harnod et al, 2008). Harnod et al. (2008) found that the epileptic group had a higher resting HR compared to healthy matched controls. This elevated HR was attributed to a lower parasympathetic drive which is thought to contribute to the higher incidence of sudden death in this group.

Retrospective analysis of EEG and ECG data during 281 seizures from 81 patients with epilepsy showed an increase in HR of ≥10 bpm in 93% of patients and an increase of ≥20 bpm occurred in 80% of patients. In 23% of seizures the tachycardia preceded the onset of the seizure by at least 3 sec (Zijlmans et al., 2002). A high incidence of tachycardic events was reported by Opherk et al. (2002) who retrospectively analysed HR and ECG changes during 102 seizures from 41 patients. Sinus tachycardia (>100 bpm) occurred in 100% of generalised seizures and 73% of partial seizures. Wilder-Smith and Lim (2001) aimed to determine if ictal HR changes varied between different races. HR was recorded in 37 non-Caucasian (Singapore-based) patients with partial seizures. 51% of seizures showed no significant change in heart rate, 22% had moderate sinus tachycardia and 11% showed severe tachycardia with an increase in HR greater than 50%.

Increased sympathetic tone has been associated with patients who later died of SUDEP. Nei et al. (2004) evaluated EEG and ECG data from SUDEP cases to investigate the potential causes of SUDEP. Twenty-one subjects (10 men, 11 women; mean age, 34.8 years) with definite or probable SUDEP were compared to patients with refractory partial epilepsy. This small clinical study found that ictal HR increases were more pronounced in the SUDEP group than the control group. In 94% of SUDEP patients sinus tachycardia occurred during or shortly after seizures, with a mean maximum HR of 149 bpm. GTC seizures within this SUDEP group were associated with the greatest increases in HR with a mean maximal HR of 169 beat/min.

Tachycardia can be potentially fatal by causing long-term myocardial damage and ventricular tachyarrhythmias. Ventricular tachyarrhythmias account for 90% of sudden cardiac deaths and are generally caused by increased sympathetic tone and structural damage (Du, 1995; Schuele et al., 2007). Tachycardia could also lead to arrhythmias such as atrial fibrillation, ST depression, QT prolongation or T wave inversion (Jansen and Lagae, 2010; Sevcencu and Struijk, 2010) which may contribute to the development of SUDEP.
4.2.3 Autonomic Nervous System’s involvement in Bradycardia and Tachycardia

Ictal changes in HR are most likely attributed to changes in autonomic function. The ANS is important in maintaining homeostasis and normal functioning of the body. It does this through a complex interaction of many brain regions; these include the NTS, nucleus ambiguus, dorsal motor nucleus of vagus, dorsal raphe nucleus, medullary reticular formation nuclei and hypothalamus (Shields, 1993; Devinsky, 2004).

KA administration is able to influence the activity of the ANS. The changes in HR seen in the present study may be due to the systemic administration of KA resulting in activation of different autonomic nuclei. Autonomic nuclei which mediate parasympathetic activity, such as the NTS or nucleus ambiguus, may be activated first. Then as seizure activity spreads or as more KA crosses the blood brain barrier it could result in stimulation of sympathetic autonomic nuclei or desensitisation of parasympathetic AMPA and KA receptors. Activation of specific autonomic nuclei with glutamatergic agonists influences the activity of the ANS and this may explain the different effects of KA on HR (Figure 4.1).

4.2.3.1 Hypothalamus

The hypothalamus is the control centre of the ANS (Shields, 1993). The dorsomedial hypothalamus (DMH) has an important role in integrating the physiological responses to stress (Da Silva, 2006). Fibres originating from the DMH innervate both sympathetic and parasympathetic nuclei such as the NTS, nucleus ambiguus, IML cell column and the rostral ventrolateral medulla (Soltis and DiMicco, 1992). The DMH also projects to the paraventricular nucleus of the hypothalamus, a site which has been implicated in tachycardia (Soltis and DiMicco, 1992).

Experiments have investigated the effect of enhanced glutamatergic transmission in the hypothalamus on autonomic control (Figure 4.1). Soltis and DiMicco (1992) examined the effect of different glutamate receptor agonists in male rats. Bilateral injections of NMDA (1-10 pmol), AMPA (0.3-3 pmol) or KA (0.1-1.0 pmol) into the DMH produced dose-dependent increases in HR. The changes in HR occurred rapidly with tachycardia produced within 45 sec. KA (1 pmol) produced the highest increase in HR, with a maximum increase in HR of 150 ± 9 bpm at 1.6 min.
The DMH projects to the paraventricular nucleus of the hypothalamus. Activation of this area is associated with tachycardia due to enhanced sympathetic activity (Soltis and DiMicco, 1992). Bilateral microinjections of KA (2 nmol) into the paraventricular nucleus in male rats resulted in an immediate and significant increase in renal sympathetic nerve activity by 53%. Renal sympathetic nerve activity gradually recovered 40-60 min post KA. Mean arterial pressure (MAP) also significantly increased by 22% compared to saline control animals (Zhong et al., 2008). This suggests that the paraventricular nucleus mediates sympathetic activity via excitatory transmission.

Autonomic function in the hypothalamus is also mediated by GABAergic transmission. Administration of the GABA$_A$ receptor antagonist, bicuculline methiodine, into the DMH results in significant increases in BP and HR in rats (Da Silva, 2006).
These experiments suggest that the DMH controls autonomic tone through a balance of inhibitory and excitatory transmission. Sympathetic activity is mediated through glutamatergic transmission while GABA_A receptor activation produces cardiac parasympathetic effects or inhibits sympathetic drive.

4.2.3.2 Nucleus of the Solitary Tract

The NTS regulates parasympathetic activity (Soltis and DiMicco, 1992; Shields, 1993). KA injected unilaterally into the NTS of anesthetised male rats produced hypotension, bradycardia and apnea (Kubo et al., 1991). This response was produced at a dose as low as 1 ng/L with maximum responses produced at 20-40 ng/μL. At 40 ng/μL there was a significant decrease in HR by 82 bpm. Within 2 min of halting KA infusion, cardiorespiratory values returned to baseline. However if the infusion was continued the cardiorespiratory effects persisted, eventually causing the animal to die. These results show that KA into the NTS decreases the cardiovagal reflex. This experiment shows that enhanced glutamate transmission mediated via activation of AMPA and kainate receptors results in enhanced parasympathetic activity. KA administered into the NTS at low doses produces bradycardia and hypotension whereas high doses (150 ng/μL) produces hypertension. This biphasic response is most likely due to the development of a depolarising block of neuronal activity. These results give further evidence to suggest that in the NTS glutamate receptors influence parasympathetic activity (Kubo et al., 1991). Activation of AMPA and kainate receptors by KA also appears to produce pronounced enhanced parasympathetic effects by decreasing renal sympathetic activity. In one study experiment microinjections of KA (2 nmol) into the NTS produced an immediate and great decrease in renal sympathetic nerve activity (~30%) and MAP (~25 mmHg) which lasted about 20 min (Duan et al., 2009).

These experiments demonstrate that the NTS is important for mediating parasympathetic activity. AMPA and kainate receptors appear to be involved in activation of the parasympathetic system as well as reducing sympathetic activity.

4.2.3.3 Nucleus Ambiguus

The nucleus ambiguus is a site of parasympathetic preganglionic neurons which projects to the heart (Soltis, 1992). Bolus microinjections of AMPA (4 pmol in 20 nl) into the nucleus ambiguus of male rats were shown to briefly (1 min) decrease MAP and HR (by 250 bpm). A dose-dependent decrease in HR and BP were observed when either AMPA (2-10 pmol) or NMDA (40-80 pmol) were administered by microinjections into the nucleus ambiguus.
Maximal response was evoked with 80 pmol NMDA which decreased HR to 100 bpm which was similar to AMPAs effect (Yan et al., 2009). This experiment showed that AMPA and NMDA delivery into the nucleus ambiguus induces fast and large HR and BP responses.

4.2.3.4 Intermediolateral column

The IML cell column in the spinal cord is where cell bodies of sympathetic pregangionic neurons are located (Soltis and DiMicco, 1992). Stimulation of the IML results in activation of sympathetic preganglionic neurons which innervate the heart, systemic arterioles and adrenal medulla, resulting in an increase in BR and HR (Shields, 1993).

When glutamate, NMDA or KA (200 pmol in 20 nL) are injected into the IML they evoked tachycardia with increases in HR of 76 ± 8, 116.5 ± 5 and 92 ± 22 bpm, respectively (Arnolda et al., 1996). Larger and more consistent tachycardiac responses were produced when glutamate was injected into the right side of the spinal cord rather than the left side (Arnolda et al., 1996).

It is possible that in the present study the large systemic dose of KA decreases the sensitivity or causes a depolarising block of kainate and AMPA receptors in the spinal cord which could explain the severe bradycardia observed. However, it is expected that if this was the case a transient tachycardic period would have occurred. Further research is required to accurately determine the effect of systemic KA in the IML.

4.2.3.4 Other Brain Regions

Other brain regions involved in controlling autonomic function include the basolateral amygdala, the cortex and the striatum. Stimulation of the basolateral amygdala results in increase in BP and decreased HR (Sevcencu and Struijk, 2010). Electrical stimulation (5-10V over 2 ms at 40 Hz) of the insular region in five patients with epilepsy (3 right side and 2 left side) resulted in changes in HR and BP. Bradycardia and depressor responses were more common than tachycardia when the left insular cortex was stimulated which suggests that there is a hemispheric difference in cortical control of HR (Oppenheimer et al., 1992).

Early studies administering KA (1 pmol in 0.5 µL) into the striatum (caudate putamen complex) of urethane anesthetised male rats found that HR and MAP increased as a consequence (Wu et al., 1984). HR reached a maximum increase of greater than 190 bpm above baseline at 50 min post KA. This tachycardic period was antagonised by prior spinal transection at C7 or bilateral vagotomy of the animal suggesting that KA induced tachycardia...
is caused by increased sympathetic efferent activity as well as decreased vagal efferent activity. More recently, Da Silva et al. (2006) found that delivery of NMDA (3-12 pmol) into the periaqueductal gray area of male rats also increased HR and BP. Maximum effect was produced by 6 pmol of NMDA where HR increased by 126 bpm above baseline. KA (1-50 pmol) also produced small non-significant increases in HR, BP and locomotion.

Goren et al. (2000) examined the influence of kainate receptors on cardiovascular responses in male rats. KA (5 and 10 pmol) injected intracerebroventricularly caused a sudden decrease in HR within 1 min which returned to baseline levels within 20 min (Goren et al., 2000). Maximum decreases of 43 bpm and 68 bpm occurred 1 min after administration of 5 pmol and 10 pmol of KA, respectively. At 10 pmol, KA produced a significant and prolonged 10 min decrease in HR following injection. Administration of 50 pmol KA however, resulted in non-significant increases in HR by 15 to 31 bpm for the 20 min observation period. Microinjections of dinitroquinoxaline (DNQX; 20, 40 and 80 pmol), a kainate receptor antagonist, failed to block the cardiovascular effect of KA. This suggests that both AMPA and kainate receptors are involved in cardiovascular responses caused by KA.

4.2.4 Glutamate Receptors and the Heart

HR changes occurred even when there was no ictal activity which suggests that systemic KA may directly affect cardiac innervation. The presence of subunit binding sites for ionotropic glutamate receptors have been found in peripheral tissues such as the pancreas, lungs, taste buds and adrenal glands (Winter and Baker, 1995; Gill et al., 1998). GluR3, NMDAR1 and KA2 subunits have been found in the brain, heart, kidney, liver and spleen (Gill et al., 1998). Immunohistochemistry has shown the presence of GluR2/3, GluR4-7, KA2 and NMDAR1 receptor subunits in the cardiovascular system. Using antibody binding studies, Gill and co-workers were able to show the presence of GluR2/3, KA2, and NMDAR1 fragments associated with ganglia cells, conducting fibres, nerve bundles and myocardiocytes in the hearts of male Sprague-Dawley rats (Gill et al., 1998). At present however, functional evidence of cardiac glutamate receptor responsiveness to excitatory amino acids remains unavailable. GluR2/3, GluR5-7, NMDAR1 and metabotropic glutamate receptors are also present in the heart of monkeys. The AMPA receptor subunit GluR1 was not present in the heart however the AMPA and kainate receptor subunits GluR2-7 where expressed in the nerve fibres of the atrium, myocardium and intramural ganglia (Mueller et al., 2003). The presence of these glutamate receptor subunits indicates that they may be involved in the regulation of impulse conduction in the heart. This suggests that in the present study KA administration may have a direct effect
on the heart. Therefore systemic KA induced seizures may not be the best model for cardiac arrhythmias during seizure activity.

Vranyac-Tramoundanas and colleagues (2008) demonstrated that domoic acid (0.05-0.25 μM) and KA (0.5-2 μM) administration to isolated rat cardiac mitochondria caused dose-dependent impairment of mitochondrial electron transport chain complexes I-V. Interestingly when domoic acid (0.05-10 μM) was administered to intact H9c2 rat cardiac myoblasts it did not compromise cellular viability. Domoic acid administration resulted in no significant changes in cell quantification, lactate dehydrogenase leakage or ROS production. This lack of damage to intact cardiomyocytes raises further questions about the toxicological effects of domoic acid and KA on the heart. These authors also examined the effect of domoic acid following systemic (2 mg/kg, i.p) or intrahippocampal (100 pmol) administration (Vranyac-Tramoundanas et al., 2011). Seizure scores were similar between groups. There was a time dependent decrease in cardiac haemodynamics (coronary flow rate, HR and LV developed pressure). The cardiac hemodynamics and myopathy did not differ between the groups, which suggests that the cardiac damage observed is a consequence of seizure-induced sympathetic stimulation rather than domoic acid acting directly on the myocardium.

Further research is required in this area to determine if the glutamate receptor subunits form functional AMPA and kainate receptor or whether the cardiac effects of KA are due to autonomic ganglia or pre/post-ganglionic fibres.

**4.3 ECG ABNORMALITIES DURING SEIZURES**

**4.3.1 ECG changes observed during KA induced seizures**

Seizure activity induced by KA altered the ECG complex. This was mainly observed in the P wave suggesting abnormal atrial depolarisation. The significant mean ECG changes which occurred during KA induced seizures are shown in Figure 4.2. In males, bradycardia was associated with significant decreases in the duration of the P wave by 30 to 40%. From 60 min post KA tachycardia started to develop which resulted in non-significant increases in the P wave amplitude by up to 50%. Tachycardia also resulted in significant increases in the QTc interval (>20% occurring 100 min post KA; Figure 4.2B and C). These results were similar to an experiment by Metcalf et al. (2009), who showed that SE induced by lithium and pilocarpine in male rats caused significant increases in BP and HR, consistent with enhanced activation of the SNS. Ten days following seizure induction, the electrical activity of the heart was altered and elevated troponin I levels and prolonged QT intervals were recorded.
Myofilament damage due to tachycardic ischaemia produced changes in electrical activity of the heart making it more susceptible to fatal arrhythmias, such as lethal ventricular tachyarrhythmias.

Bradycardia in females was associated with a significant decrease in P wave amplitude and QTc interval by 30% (Figure 4.2D). Tachycardia was accompanied by large increases in the S amplitude, with increases of greater than 2-fold being recorded (Figure 4.2E). KA induced seizure activity in females resulted in significant increases in the PR interval by 30% 40 min post KA which lasted for 2 hours. This change was independent of HR. A prolonged PR interval as well as a decrease in P wave amplitude indicates the occurrence of premature atrial beats (Stouffee, 2009). An increase in the S wave amplitude as seen during tachycardia in females can be caused by ventricular hypertrophy (Figure 4.2; Khan, 2008).

The main ECG abnormalities observed were alteration in the P wave or skipped beats (Figure 3.14; Table 3.1). Abnormal depolarisation of the atria occurred in all animals. This generally consisted of decreases in the amplitude of the P wave although P wave inversion and shortening were also observed. The P wave represents electrical discharge from the SA node and the contraction of the atria (Kubdu et al., 2000; Stouffee, 2009; Catalano, 2002). Changes in the P wave can be caused by tissue damage or electrolyte abnormalities. The decrease in

![Figure 4.2: Mean ECG changes which occurred during following KA-induced seizures (10 mg/kg) in Sprague-Dawley rats. Blue lines represent significant increases in ECG variable. Red lines represent significant decreases in ECG variable. A) Normal rat ECG. B) Male ECG during bradycardia. C) Male ECG during tachycardia. D) Female ECG during bradycardia. E) Female ECG during tachycardia.](image-url)
duration observed in males during the bradycardic period may be caused by hypokalemia while hyperkalemia could cause the decrease in amplitude of the P wave in females (Stouffee, 2009). A decrease in the amplitude of the P wave can also be caused by atrial hypertrophy. However due to the rapid onset and short duration of this arrhythmia it is most likely a result of abnormal electrophysiology. The absence of P waves, as seen in five of the nine rats, can be due SA block and AV junctional rhythms (Khan, 2008).

The PR interval is used to measure the conductance time through the AV node. The PR interval can be prolonged by factors which slow conductance through the AV node such as ischaemic injury, fibrosis of the node or reduced sympathetic stimulation (Marsh et al., 2008; Stouffee, 2009). The PR interval can also be lengthened as a consequence of increased vagal tone. This suggests that in females seizure activity enhances parasympathetic stimulation. This is supported by studies which have found that oestrogen administration results in decreased sympathetic stimulation and enhance parasympathetic stimulation (Saleh et al., 2000b; Saleh and Connell, 2003). During seizure activity this may protect the heart from sympathetic induced cardiac damage.

The QT interval represents the depolarisation and repolarisation of the ventricles. It is used as a clinical index when assessing primary cardiac arrest in heart disease (Whitsel et al., 2001). Changes in the QT interval suggest potentially fatal alterations in ventricular repolarisation. A prolonged QT interval is an established risk factor for life-threatening ‘Torsade de Pointes’ tachycardia and sudden death (Shimizu and Antzelevitch, 1998; Surgers et al., 2010). Even a transient increase as observed during epileptic discharge can predispose a patient to ventricular fibrillation and sudden death (Tavernor et al., 1996). Prolongation of the QT interval can also be caused by enhanced sympathetic stimulation and abnormal channel functioning such as inhibition of outward K^+ currents or enhancement of inward sodium or calcium currents (Shimizu and Antzelevitch, 1998; Yan et al., 2003; Surges et al., 2009). A shortening of the QT interval can be caused by hyperkalemia, hypercalcemia or acidosis (Surges et al., 2010). This rapid repolarisation can facilitate re-entrant excitation leading to fatal arrhythmias such as atrial fibrillation (Surges et al., 2009).

KA administration produced a strong correlation between P wave amplitude and HR. Interestingly this effect did not appear to be present in the saline control animals. In contrast to these findings, Hinds et al. (1972) found that as HR decreased the P wave amplitude increased in dogs. The amplitude of the P wave varied depending on the position of the ECG lead. No general relationship could be found between HR and P wave amplitude. It is possible that alterations in electrophysiology or catecholamine levels induced by seizure activity is
implicated in this effect, however more research is required to determine if this correlation is causally related.

4.3.2 Ictal ECG abnormalities in Humans

Ictal ECG abnormalities have been reported during seizure activity. These changes are generally benign but potentially fatal changes (such as ST depression and T wave inversion) occur in 6 to 13% of seizures (Devinsky, 2004). ECG abnormalities are more common in refractory epilepsy, where up to 44% of patients have been reported to have at least one rhythm or repolarisation abnormality during or shortly following a seizure (Stollerberger and Finsterer, 2004). These include AV block, atrial fibrillation, supraventricular tachycardia, ventricular premature depolarisation and bundle branch block (Devinsky, 2004).

Interictal ECG data has demonstrated that epileptic patients have a faster ventricular rate and longer QT interval compared to controls. Complex partial and secondary generalised seizures are associated with the greatest increases in HR (Drake et al., 1993). Opherk et al. (2002) retrospectively analysed HR and ECG changes during 102 seizures from 41 patients. ECG abnormalities occurred in 22% of seizures (37% of patients). Most arrhythmias were benign (such as premature atrial/ventricular depolarisations, AV block and sinus arrhythmias) but in 10% of patients potentially serious changes occurred. Ictal HR increases and ECG abnormalities were more common in generalised seizures than partial seizures, with 13% of seizures associated with potentially fatal arrhythmias. Similar results were reported by Zijlmans et al. (2002) who retrospectively analysed 281 seizures from 81 patients with epilepsy who had prolonged EEG and ECG recordings. In 50% of patients ECG abnormalities occurred during or directly following a seizure. Ictal ECG abnormalities included ST elevation/depression, AV block and T wave inversion. Potentially severe abnormalities occurred in 14% of patients and one of these was an asystole which lasted for 30 sec. TLE is associated with a high incidence of benign arrhythmias, reported to occur in nearly 60% of patients (Surgers et al., 2010). In 68% of temporal lobe seizures a shortening of the QT interval occurs. Shortening of the QT interval was more common in females and patients with right sided seizure onset. Prolonged QT interval only occurred in 6% of temporal lobe seizures. Interestingly this data supports the present animal study where KA induced temporal seizures were associated with a high incidence of QTc shortening which was more common in females than males.
The ECG abnormalities reported interictally and ictally in epileptic patients was similar to data analysed from SUDEP cases. Nei et al. (2004) evaluated EEG and ECG data from SUDEP cases to investigate the potential causes of SUDEP. Ictal rhythm or repolarisation abnormalities occurred in 56% of cases. These included premature ventricular depolarisation, atrial fibrillation, ST elevation and right bundle branch block. Tavernor et al. (1996) compared 11 SUDEP cases to age and sex matched patients who had tonic-clonic seizures. The QT interval was significantly longer during seizure activity than interictal periods. However there was no significant difference between the groups. Prolongation of the QT interval may be due to the surge in sympathetic activity which is reported to occur during seizures.

Ion channel malfunctioning leading to ECG arrhythmias can be hard to identify during an autopsy so cannot be ruled out as a contributor to SUDEP (Leung et al., 2006). ECG abnormalities can lead to two main potentially fatal arrhythmias, ictal ventricular tachyarrhythmias and ictal bradycardia/asystole (Schuele et al., 2007). These can cause cerebral hypoperfusion and global hypoxia resulting in sudden death.

### 4.4 PATHOLOGY

#### 4.4.1 Cardiac Morphology

The most common cardiac morphological feature observed following KA administration in rats was interstitial oedema which was observed in the subendocardium and septum of all ventricular planes. Perivascular infiltration of non-specific inflammatory cells and multifocal degeneration of muscle fibres typical of myocardial necrosis were also observed within this region. Nuclear vacuolisation, an early marker of reversible ischaemic damage, occurred in these KA dosed hearts (Tsang et al., 2008a). Intra-myocardial haemorrhage or infiltration of red blood cells into the muscle fibres was present in the two female rats which exhibited the highest seizure activity. These pathological changes induced by KA seizures are comparable to clinical data which demonstrates that seizure activity is associated with reversible structural cardiac damage.

Repetitive seizure activity is hypothesised to activate the ANS leading to structural heart damage which increases the heart’s susceptibility to cardiac arrhythmias or ischaemia (Kloster and Engelskjon, 1999; Jansen and Lagae, 2010). Hearts from SUDEP patients are generally dilated and heavier than controls. Non-fatal pathological changes have been reported in 33% of cases (Kloster et al., 1999; Stollerberger and Finsterer, 2004). Fibrosis of the walls of small coronary arteries, interstitial myocardial fibrosis, atrophy of cardiomyocytes, myofilament
degeneration, subendocardial fibrosis, leukocytic infiltration and oedema of conductive tissue has been found which could contribute to SUDEP (Stollerberger and Finsterer, 2004). Natelson et al. (1998) found pathological changes present in five out of seven hearts from epileptic patients. Four hearts had evidence of irreversible perivascular and interstitial fibrosis and all had reversible myocyte vacuolisation, predominantly in the subendocardium (Natelson, 1998).

Enhanced sympathetic activation or decreased protective vagal reflexes are associated with ventricular arrhythmias and sudden cardiac death (Vaseghi and Shivkumar, 2008). This appears to be implicated in the cause of SUDEP and may explain the cardiac damage observed in rats 48 hours post KA. Repeated hypoxemia and increased catecholamines can cause structural heart damage making the heart more susceptible to fatal arrhythmias, increasing the risk of sudden cardiac death.

4.5 GENDER DIFFERENCES

4.5.1 Gender Differences

Many gender differences were observed in this experiment. Females exhibited higher and longer lasting seizure behaviours and these were associated with increased power spiking of theta, delta and alpha frequency bands. Males exhibited a high level of WDS following KA administration. Females also exhibited more severe changes in HR and significant increases in the PR interval. Massive decreases in the P wave amplitude and shortening of the QTc interval were observed in females while males presented with significant decreases in the P duration which were not observed in females. In general, the pathological damage did not appear to be different between animals except in two females where histology revealed cardiac haemorrhage.

A similar experiment with contrasting results was performed by Mejias-Aponte et al. (2002). KA (10 mg/kg, i.p.) seizures were induced in Sprague-Dawley rats and behaviour was observed for 3 hours. Females had a shorter latency to mild and full limbic convulsions but males exhibited a higher frequency of full limbic seizures. The authors attributed these gender differences to testosterone levels, as testosterone treatment increased seizure susceptibility.

The increased seizure severity observed in females in this study is particularly interesting. Clinical data has suggested that oestrogen may have pro-convulsant effects. However experimental data shows that oestrogen treatment enhances GABAergic transmission by increasing GAD activity in neurons, inducing GABA release in the hippocampus and up-regulating GABA_A receptors (Saleh and Connell, 2003). These results suggest that elevated
oestrogen levels in females should protect the hippocampus from KA and reduce its ictogenic effect due to elevated inhibitory transmission.

### 4.5.2 Oestrogen

Oestrogen mediates its effects through both genomic and non-genomic mechanisms. Activation of nuclear ERs initiates of gene transcription, the effects are delayed (minutes to hours) and long lasting (Zhou *et al.*, 2001; Veliskova, 2006). The gene expressed depends on the cell type and the rate of transcription can be influenced by the presence of promoters or repressors (Smith, 1997). ER can also be membrane bound. These receptors act via second messenger systems or direct coupling to ion channels such as activation of cAMP and mitogen-activated protein kinase pathways and modulate Ca$^{2+}$ influx (McEwen, 2002; Veliskova, 2006; Veliskova, 2007). Other ER mediated effects include a decrease in neuronal NOS, blockade of VGCC and direct inhibition of NMDA receptor (Veliskova, 2007).

#### 4.5.2.1 Oestrogen and Seizures

At present, there are controversial results about oestrogen’s effect on seizures. Some studies suggest that there is a decrease in seizure rate around ovulation while others report a lower seizure threshold with high levels of circulating oestradiol (Veliskova, 2006; Veliskova, 2007). Endocrine reproductive disorders are often reported in women with TLE. Restoring normal ovulatory cycles with oestrogen therapy has been found to be an effective seizure therapy in these patients (Veliskova *et al.*, 2000; Veliskova, 2006).

In women, seizure frequency can change in relation to the menstrual cycle, this is called catamenial epilepsy. Seizure frequency generally increases during or just before mense, this has been linked to an increase in the oestrogen/progesterone ratio. Oestrogen has been linked to pro-convulsant effects while progesterone is thought to be anti-convulsant. However other mechanisms may be involved such as pH changes, electrolyte or mineral imbalances or a decrease in AED concentration (Veliskova, 2007). No clear connection has been made between the contraceptive pill and seizure activity. A majority (~50%) of studies suggest that oestrogen is anti-convulsant while others show no therapeutic value or worse, a pro-convulsant effect (Veliskova, 2007). Hormone replacement therapy in post-menopausal women has been associated with a decrease in seizure frequency (Pebbles *et al.*, 2000).

The different effects of oestrogen may be attributed to the treatment regime. In ovariectomised rats pre-treatment with physiological doses of oestradiol (5-10 μg) can delay KA induced
seizure onset, whereas 20 µg produced no effect and 40 µg was pro-convulsant (Veliskova, 2006; Veliskova, 2007). Low doses of oestrogen have been reported to increase seizure threshold while high doses are pro-convulsant. Chronic treatment is more beneficial than acute and is associated with anti-convulsant effects (Veliskova, 2007). During chronic treatment oestrogen may mediate neuroprotective effects by activating growth factors and providing an antioxidant action. This would be beneficial in preventing long term damage, by reducing seizure severity and the risk of developing SUDEP.

β-oestradiol can alter neuronal activity by directly interacting with NMDA, AMPA or KA receptors (Veliskova, 2007). Oestrogen can change the subunit composition of NMDA receptors and directly interacts with NMDA receptors to inhibit activity or enhance ligand binding (Veliskova et al., 2000; Veliskova, 2007). The effect produced may depend on the receptor composition or the brain region. Oestradiol also increases muscimol binding in the hippocampus and increases GABA production (Veliskova, 2006; Veliskova, 2007). The oestradiol-induced enhancement of the GABAergic system seems to compensate for the increase in NMDA and AMPA receptor mediated effects (Veliskova, 2007). Oestrogen activates multiple cellular components, such as signalling pathways, growth factors or anti-apoptotic molecules which are implicated in neuronal survival (Datta et al., 1997; Garcia-Segura et al., 1998; Brunet et al., 2001; Kuroki et al., 2001; D’Astous et al., 2005; Bourque et al., 2007). Furthermore, oestrogen stimulates the secretion of growth factors (such as neurotrophins, brain derived neurotrophic factor and nerve growth factor) which produce neuroprotective effects (Miranda et al., 1996; Son et al., 1999). The results imply that oestrogen has a neuroprotective effect in the CNS which decreases seizure induced brain damage. Although females in this study experienced more severe seizure activity it is possible that there is less brain damage, however further experiments are required to investigate this.

The antioxidant ability of 17β-oestradiol is produced due to the presence of the phenolic A ring of the steroid. The phenolic A ring is a potent electron donor and free-radical scavenger which prevents lipid peroxidation-induced membrane damage (Moosmann and Behl, 1999; Veliskova et al., 2000). This could reduce cell death caused by neurotoxins, such as KA and domoic acid, especially since these are associated with increased production of ROS and induction of apoptosis through mitochondrial damage (D’Astous et al., 2005). Oestrogen has also been found to block VGCC and can decrease neuronal NOS (Veliskova et al., 2000), which can reduce excitotoxicity during seizure activity.

The results from these experiments suggest that oestrogen should evoke beneficial effects in the present study rather than the enhanced seizure activity which was observed. One potential
reason for the adverse effect observed could be due to the acute seizure induction paradigm employed with KA. Oestrogen has been reported to potentiate the effect of KA currents in the hippocampus by increasing the density of NMDA receptors in CA1 region of the hippocampus (Gu et al., 1999). However another experiment found that oestrogen treatment in ovariectomised rats protected the hippocampus from KA induced damage (Veliskova et al., 2000). Galanopoulou et al. (2003) compared the effects of 17β-estradiol (2 mg/rat, s.c. for 4 days) in adult male and ovariectomised female rats prior to lithium-pilocarpine-induced SE. Oestrogen treatment did not alter the onset of first clonus in ovariectomised rats but accelerated it in males. Oestrogen treatment resulted in a decrease in hippocampal damage in males and females. It is possible that the presence of oestrogen in female rats acts to potentiate the immediate seizure activity induced by KA but may reduce the onset of recurrent seizures and reduce the neuronal damage induced by seizure activity.

4.5.2.2 Cardioprotective Effects of Oestrogen

Females tend to be at a lower risk of developing cardiovascular diseases (Du et al., 1995). Clinical studies in post-menopausal women have shown that oestrogen replacement therapy can significantly decrease the progression of cardiovascular disease and increase the degree of recovery following the onset of a cardiovascular pathology (Saleh et al., 2000d). It can reduce the risk of post-menopausal morbidity and mortality of cardiovascular disease by about 50% (Du et al., 1995). Clinically, it has been demonstrated that women with cardiovascular disease have significantly lower serum oestrogen concentrations compared to healthy age-matched controls (Hanke et al., 1997). Oestrogen has many cardioprotective effects which may relate to its ability to increases plasma levels of high density lipoprotein, inhibit peroxidation of lipoproteins and inhibit atherosclerotic plaque formation (Du et al., 1995). Oestrogen has also been shown to produce vasodilation (Du et al., 1995), suggesting that it may suppress the vasoconstrictor response of coronary arteries to increased sympathetic tone. As indicated earlier, oestrogen has been indicated to have antioxidant properties, therefore enhancing free-radical scavenging and decreasing lipid peroxidation which may protect the heart from seizure induced ischaemic damage.

Presynaptic feedback of the sympathetic nervous system is mediated by central α2 adrenoreceptors (Du et al., 1995). This inhibitory pathway is more potent in female rats than males during normal and acute ischemic conditions (Du et al., 1991). Oestrogen has been reported to increase the density and activity of α2 adrenoreceptors resulting in reduced NA release (Janes et al., 1983; Morita et al., 1987). Males have higher levels of NA during
stressful conditions than females (Du et al., 1995), which could lead to enhanced cardiac damage during seizure activity.

The cardioprotective effect of oestrogen could explain why SUDEP is more prevalent in males. The elevated oestrogen levels in females may act to reduce ischemic damage caused during seizures, reducing the susceptibility of the heart to arrhythmias.

4.5.2.3 Oestrogen and the Autonomic Nervous System

The gender differences observed in this experiment and clinical studies may also be mediated by the effect of oestrogen’s in the ANS. Oestrogen can modulate the activity of the ANS centrally by acting at specific autonomic nuclei. Oestrogen enhances parasympathetic activity by increasing ACh release and increasing choline uptake causing enhanced neuronal cholinergic transmission and vagal activity (Veliskova, 2007). Females are reported to have a higher content of ACh and enhanced activity of choline acetyltransferase compared to males. This is further increased by oestrogen treatment and decreases following ovariectomy (Du et al., 1995). Oestrogen decreases sympathetic tone by increasing the density and enhancing the function of presynaptic α2 adrenoreceptors (Du et al., 1995). These results show that the parasympathetic system is more predominate in females which may result in less seizure induced cardiac damage.

In previous experiments it has been shown that cardiovascular autonomic function is mediated by nuclei in the brain stem, these include the NTS, parabrachial nucleus, central nucleus of the amygdala and the IML of the spinal cord. Oestrogen has been shown to centrally modulate autonomic tone (Figure 4.3). Saleh et al. (2000d) found that systemic oestrogen treatment resulted in dose-dependent increases in parasympathetic tone while decreasing sympathetic tone. Maximum effect was observed at 1x10⁻² mg/ kg and this dose was then used in further experiments (Saleh et al., 2000d). Female Sprague-Dawley rats were ovariectomised and oestrogen replaced (5 μg/kg, s.c. for 7 days) to produce a stable oestrogen level similar to that present at proestrus. A bolus injection of oestrogen (1x10⁻² mg/kg, i.v.) resulted in a significant increase in vagal nerve activity and a decrease in renal nerve activity as recorded in anesthetised animals (Saleh and Connell, 2000). There was a 7-fold increase in vagal nerve activity 30 min following the oestrogen injection, which was associated by a decrease in HR by 55 bpm. Treatment with ICI-182780 (1 pM, 50 nL per side), an oestrogen receptor antagonist, into the nucleus ambiguus blocked the increase in parasympathetic nerve activity. ICI-182780 was also injected into the intrathecal space to determine if ER inhibition influenced sympathetic preganglionic neurons of the IMC. ICI-182780 (1 pM, 10 μL) injected into the
intrathecal space prevented the inhibitory effect of oestrogen on sympathetic activity, demonstrating that the activation of central ER acts to decrease sympathetic activity as well as enhance parasympathetic effects.

Saleh et al. (2000b) also examined the effect of centrally administered oestrogen in oestrogen replaced (5 μg/kg, s.c. for 7 days) ovariectomised rats. Oestrogen replacement resulted in a lower baseline HR of 298 bpm compared to 329 bpm in the saline treated ovariectomised rats. Oestrogen (0.5 μM; 100nL/side) into the NTS, rostral ventrolateral medulla, parabrachial nucleus, central nucleus of the amygdala and the intrathecal space resulted in a significant decrease in renal nerve activity and MAP. Injection of oestrogen into the NTS, nucleus ambiguus, parabrachial nucleus and the intrathecal space resulted in decreases in HR and decreased vagal parasympathetic nerve activity. Injection of oestrogen into the insular cortex also resulted in a decrease in renal sympathetic nerve activity. These results show that oestrogen mediates its effect by reducing sympathetic activity and enhancing parasympathetic activity. Similar changes were observed in male Sprague-Dawley rats (Saleh et al., 2000a; 2000c). Oestrogen (0.5 μM; 100 nL/side) administered into the NTS, nucleus ambiguus and intrathecal space resulted in enhanced parasympathetic activity as measured by a significant increase in efferent vagal nerve activity. Renal sympathetic nerve activity was significantly depressed by oestrogen administered into the NTS, rostral ventrolateral medulla and intrathecal space (Saleh et al., 2000a; 2000c). BP and HR significantly decreased by 10 mmHg and 20 bpm at 30 min following administration of oestrogen into the NTS, whereas injection of oestrogen into the nucleus ambiguus resulted in decreased HR but no change in BP (Saleh et al., 2000c). These results suggest that activation of ERs decreases BP through inhibition of sympathetic activity and decreases HR by activating the parasympathetic system.

Oestrogen’s effects have been linked to ER-mediated influence on excitatory and inhibitory transmission. Xue et al. (2003) used electrophysiological experiments to determine the effect of oestrogen in the NTS of ovariectomised rats. 17β-oestradiol application inhibited the firing of 68% of neurons in the NTS. This effect was modulated by the ER as ICI182780 blocked the inhibitory effect of oestrogen. In 16% of neurons oestrogen treatment resulted in an increase in the firing rate of NTS neurons from 2.6 to 7.3 Hz. This inhibition was not provoked by enhanced GABA receptor activity as bicuculline and phaclofen did not prevent the effect of oestrogen. The excitatory effects of L-glutamate, AMPA and NMDA were significantly inhibited by the presence of oestrogen producing further evidence to support an anti-convulsant role for oestrogen.
The consequence of oestrogen on NMDA and GABA A receptors was examined in male Sprague-Dawley rats. Administration of oestrogen (0.5 μM; 100 nL/side) into the parabrachial nucleus resulted in a decrease in HR by 22 bpm corresponding to a significant reduction in sympathetic tone while increasing parasympathetic tone by 34% at 30 min post-oestrogen. Co-administration of 3-(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid (10 μM, an NMDA receptor antagonist) completely blocked oestrogen-induced HR and vagal parasympathetic nerve activity changes. Co-injection of bicuculline (0.1 μM, GABA A receptor antagonist) inhibited oestrogen-induced decrease in BP and sympathetic nerve activity (Saleh and Connell, 2003). The results from this experiment demonstrate that oestrogen can alter autonomic tone through inhibitory and excitatory modulation. Oestrogen may act via GABA A receptor activation to decrease BP and renal nerve activity while NMDA receptors may be involved in decreasing HR and vagal nerve activity.

The different effects mediated by oestrogen are most likely due to activation of different receptor subtypes. Following oestrogen administration, parasympathetic nerve activity is enhanced preceding alterations in sympathetic nerve activity (Saleh et al., 2000c; 2000d). This difference may be due to the differential distribution of ER subtypes in autonomic ganglion. ERα is present in a high concentration in the nucleus ambiguus which is a site of parasympathetic modulation while the ERβ is found in the IMC a site of preganglionic neurons (Saleh et al., 2000d). Both receptor subtypes are present in the NTS and parabrachial nucleus (Saleh and Connell, 2003) where they mediate autonomic control by enhancing vagal nerve stimulation and decreasing renal nerve activity (Saleh et al., 2000b; 2000c). It is also possible that the early onset changes are mediated by non-genomic mechanisms while alterations in sympathetic nerve activity occurring 60 min post-oestrogen administration are due to genomic effects. The variability in ER effects may also be mediated by the ER interaction with other channels in different cell types. Stimulation of the ER could result in modulation of K+, Ca2+ or GABA A receptors depending on which channel type it is coupled to.
Progesterone should not be dismissed as a hormonal modulator of seizure activity. In females progesterone is present in high levels during the second half of the menstrual cycle or during pregnancy. Small amounts are also present in males where it is secreted from the testes. An increase in seizure frequency during menses may be a result of decreased progesterone levels (Veliskova, 2007). Consequently, the beneficial anti-convulsant effects attributed to contraceptive pill use and hormone replacement therapy may be due to the replacement of progesterone levels (Pebbles, 2000).

Progesterone potentially produces pro-convulsant effects because it increases glutamate binding in the hypothalamus of female rats (Diano et al., 1997). However its metabolite, allopregnaolone (3α-hydroxy-5α tetrahydroprogesterone), produces anticonvulsant effects by potentiating the activity of GABA A receptors (Smith et al., 2002). Progesterone receptors are present in autonomic nuclei which regulate cardiovascular control, such as the medial nucleus of the amygdala, preoptic suprachiasmatic nucleus and periventricular hypothalamic nucleus.

Figure 4.3: Summary of published effects of oestrogen administration on specific autonomic nuclei. Brain adapted from Iverson et al. (2000)
(Stumpf, 1990). Progesterone is important for regulating cardiac function which may have a protective role during seizure activity.

4.5.4 Testosterone

Testosterone is an androgen hormone. A decrease in serum testosterone levels is associated with a decrease in muscle mass and bone density as well as neurological effects such as dementia, depression and anxiety (Hammond et al., 2001; Gray et al., 2005). Testosterone mediates its effects via genomic and non-genomic mechanisms. Binding of testosterone to nuclear androgen receptors modulates gene transcription (Hammond et al., 2001). Testosterone is metabolised to two neurosteroids, 3α-androstenediol (3α-diol) and 17β-oestradiol (Figure 4.4). 5α-reductase converts testosterone to the intermediate 5α-dihydrotestosterone (DHT) which is further reduced to 3α-diol (Reddy, 2004). Testosterone can also be converted to 17β-oestradiol by aromatase where its effects are produced by binding to ERs (Reddy, 2004; Bolour and Braunstein, 2005). Both 3α-diol and 17β-oestradiol are synthesised in peripheral tissues and by glial cells in the brain (Reddy, 2004). DHT has higher biological activity at the androgen receptor than testosterone (Henderson et al., 2006). Androgen receptors are located throughout the brain where they mediate cardiac function. They are present in autonomic nuclei such as the dorsal motor nucleus of the vagus, nucleus ambiguus, basal hypothalamus and amygdala (Stumpf, 1990).

4.5.4.1 Testosterone and Seizures

Males have a higher risk of developing seizure induced brain damage and the male gender is associated with an increased risk of developing SUDEP. This suggests that the presence of testosterone or absence of oestrogen may be involved in this pathology (Nilsson et
Reduced testosterone levels have been observed in males epileptic patients suggesting testosterone has an anti-convulsant effect (Mejias-Aponte et al., 2002). However it has also been proposed that testosterone increases seizure activity by decreasing GABA levels in various brain regions (Lasaga et al., 1988; Gray et al., 2005). Androgen and AMPA receptors are colocalised in many brain regions (Diano et al., 1997). Testosterone produces a stimulatory influence on the expression of AMPA receptors in the hypothalamus (Diano et al., 1997) suggesting testosterone may be instrumental in mediating the pro-convulsant effects, especially in patients with TLE.

KA administration (10 mg/kg, i.p.) in Sprague-Dawley rats revealed gender differences in latency and frequency of seizure activity (Mejias-Aponte et al., 2002). Males exhibited higher seizure frequency and testosterone treatment in gonadectomised or intact males produced an increase in seizure frequency and severity. These results contrast to the gender differences observed in the present experiment as they demonstrate that in the KA model of TLE testosterone decreases seizure threshold.

Systemic administration of testosterone (10-200 mg/kg, s.c.) in male Sprague-Dawley rats produced a dose-dependent decreased in pentylenetetrazol seizure threshold. A dose of 200 mg/kg caused a 25% decrease in seizure threshold (Reddy, 2004). It is possible that the seizure facilitating effects of testosterone are triggered by increased synthesis of 17β-oestradiol. Letrozole, an aromatase inhibitor, significantly prevented testosterone induced decreases in seizure threshold. Seizure susceptibility was greatest in rats with low 3α-diol and high 17β-oestradiol, demonstrating that the pro-convulsant effects of testosterone are produced by the conversion of testosterone to 17β-oestradiol. This experiment also suggested that 3α-diol mediates the anti-convulsant properties of testosterone, most likely by allosterically enhancing the activity of the GABA_A receptor (Reddy, 2004). Smith et al. (2002) found that hippocampal slices from males exhibited significantly greater excitatory post-synaptic potentials than females. Circulating levels of testosterone were positively correlated to increased excitatory transmission, producing a pro-convulsant effect in the hippocampus. Removal of sex hormones by gonadectomy reduced the excitatory transmission in males but not females, suggesting a gender dependent effect. In the hippocampus, the effect of testosterone appears to be mediated by decreased activation of the GABA system or enhanced responsiveness to the excitatory system in the CA1 region of the hippocampus. As previously proposed the negative effects of testosterone may be mediated through its metabolites oestradiol and 5α-dihydrotestosterone. In an amygdala kindled seizure model, testosterone and its metabolites enhanced the development of seizures (Edwards et al., 1999). Testosterone itself reduced the time it took for generalised
secondary seizures to develop, while oestradiol produced the most potent ictotogenic effect, indicating that antagonism of ER in males may be protective.

Testosterone has also been reported to enhance GABAergic transmission. Testosterone treatment in orchidectomised male rats produced an anxiolytic effect through potentiation of GABA<sub>A</sub> receptor activity. However, this beneficial effect was only observed following chronic treatment (Fernandez-Guastia et al., 2005). The anti-convulsant properties of testosterone may be mediated through its metabolites. 3α-diol allosterically enhances the activity of GABA<sub>A</sub> receptors producing a pronounced increase in Cl<sup>-</sup> influx (Reddy, 2004).

4.5.4.1 Cardioprotective Effects of Testosterone

Gender differences have been observed in cardiac function. Men have a higher incidence of coronary heart disease, myocardial infarction and sudden death (Cavasin et al., 2003; Tsang et al., 2007). However there is also evidence suggesting that testosterone has cardioprotective effects. Testosterone can decrease ischaemic Ca<sup>2+</sup> overload, activate mitochondrial K<sub>ATP</sub> channel flux and increase responsiveness to NA (Crews and Khalil, 1999; Tsang et al., 2007) which can reduce further cardiac damage. Testosterone has a positive inotropic effect on the heart by activating androgen receptors which enhances stimulation of α1 and β1 adrenoreceptors (Tsang et al., 2009). Testosterone also enhances contraction of the myocardium by increasing Ca<sup>2+</sup> release via the ryanodine receptor-activated channels in the sarcoplasmic reticulum (Tsang et al., 2009). These effects can improve contractility post ischaemic injury.

Tsang et al. (2008b) studied isolated perfused hearts from orchidectomised and normal male rats in order to determine the effect of testosterone on cardiac function. Orchidectomy resulted in significant reductions in body and heart weights compared to sham animals. The isolated hearts underwent regional ischaemia and re-perfusion with NA to mimic sympathetic over-activation. The infarct size was significantly larger in the orchidectomised males and testosterone replacement protected the heart from further damage. The beneficial effect of testosterone was mediated through activation of α1 adrenoreceptors. Testosterone increased the expression of α1 and β1 adrenoreceptors. These effects were mediated by the androgen receptor as the effect was blocked by cyproterone acetate an androgen receptor antagonist.

The detrimental effect of testosterone may be due to enhanced myocyte hypertrophy mediated by cardiac androgen receptors (Cavasin et al., 2003; Tsang et al., 2008b). Enhanced apoptosis caused by activation of androgen receptors and indirect stimulation of β1 receptors could also
increase cardiac damage post ischaemia (Cavasin et al., 2003; Tsang et al., 2008b). Testosterone enhances the expression and activity of β1 receptors which can increase heart damage (Tsang et al., 2008b). The cardiac effects mediated by testosterone appear to be mediated by genomic and non-genomic androgen receptor mechanisms (Cavasin et al., 2003).

MI induction in male mice produced a higher incidence of cardiac rupture, decreased ejection fraction and left ventricular dilation compared to females (Cavasin et al., 2003). This effect appears to be mediated by sex hormones because testosterone treatment in female mice resulted in a decrease in cardiac ejection and increased left ventricle size following MI, whereas oestrogen treatment or castration in males prevented deterioration of cardiac function and remodelling. There was also an increase in mortality post MI in males and testosterone treated females. These results demonstrated that oestrogen was cardioprotective while testosterone enhanced cardiac dysfunction.

SUDEP may be more common in males due to the cardioprotection associated with oestrogen. In males, repetitive seizure activity with enhanced sympathetic stimulation could expose the heart to recurring ischemic damage resulting in increased mortality.

### 4.6 LIMITATIONS AND FUTURE STUDIES

#### 4.6.1 Animal Model

A major limitation of this study is systemic administration of KA. It is unknown whether the bradycardic effect observed occurred due to KA acting directly on the heart or through centrally mediated effects. Systemic administration was used due to time constraints. Previous work in our lab demonstrated that the cardiac damage observed was due to epileptic discharge. Domoic acid was administered systemically (2 mg/kg, i.p.) or intrahippocampally (100 pmol) (Vranyac-Tramoundanas et al., 2011, in publication). Seizure score and cardiac damage did not significantly differ between groups suggesting that the cardiac damage observed was a consequence of seizure-induced sympathetic stimulation rather than domoic acid directly acting on the myocardium. Although KA and domoic acid both bind to AMPA and kainate receptors it is possible that KA produces other cardiac specific effects which have not been observed by domoic acid. It would also be interesting to examine the effect of seizure activity in specific autonomic nuclei as well as generalised seizure activity.

In future experiments it would be beneficial to use a longer duration animal model as HR and behavioural activity returned to baseline within 6 hours post KA. Using an animal model where
recurrent seizure activity occurs would allow for long term cardiac and neuronal damage to be observed.

4.6.2: Cardiac Function

Examining cardiac histology over a longer time frame would help understand the progression of cardiac damage which occurs following KA administration. Vranyac-Tramoundanas et al. (2011, in publication) found that cardiac damage was still present 14 days post domoic acid administration. Analysis of troponin I and creatinine kinase levels would allow for further quantification of cardiac damage. Changes in the ECG, especially the P wave, can occur due to alterations in catecholamine level and electrolytes. Future experiments measuring serum NA and K⁺ levels would help understand the cause of the observed ECG abnormalities and help to confirm if seizure activity does result in a sympathetic storm.

Immunohistochemistry would also help determine if there are functional glutamate receptors and if these contribute to the cardiotoxic effects of KA. Westerns blot analysis, immunohistochemistry, immunoprecipitation and radioligand binding can be used to determine the subunits of glutamate receptors in the heart. Administration of specific glutamate receptor agonists and antagonists for each receptor subtype in an intact isolated heart and in collagenase digested cardiomyocytes would also determine if these receptors were functional.

4.6.3 Oestrogen and Testosterone

In further experiments, it would be important to measure both oestrogen and testosterone levels in intact animals. These levels could then be correlated to seizure activity and heart damage to determine which hormone may be mediating the beneficial or negative response. It may be that elevated oestrogen levels have a pro-convulsant effect but also reduces the cardiac damage which occurs. In the present study there was no significant difference in cardiac variables between animals. However Female #1 exhibited extremely high seizure behaviours compared to the other animals, with 26 tonic-clonic convulsions recorded which may have been due to elevated oestrogen levels.

Another option of controlling hormone levels is to ovarectomise the female rats and hormone replace to control oestrogen levels. The oestrous cycle in rats is divided into proestrous, oestrus, metestrous and diestrus. Oestrogen levels are highest during proestrous (50 pg/ml) (Xue et al., 2003) and this can be achieved through administering oestradiol at 5 μg/kg for 7 days (Saleh et al., 2000b). Ovariectomising the rat will simplify the model and allows for better
interpretation of oestrogen effect. However using intact rats means that the effect of endogenous oestrogen can be examined but it would be important to ensure oestrogen levels were recorded prior to seizure induction.

4.7 CONCLUSIONS

The data from this study supports clinical studies examining ECG abnormalities and structural heart damage which occurs during seizure. Seizure activity causes alterations in cardiac function by influencing the activity of the autonomic nervous system, which may be the cause of SUDEP in susceptible individuals. Although SUDEP has not been associated with a single risk factor, a combination of these events may make some patients more vulnerable to developing SUDEP. Structural cardiac damage leading to ventricular tachyarrhythmias and asystole appear to be the main cause of sudden death in epileptic patients.

In this animal model, systemic administration of KA resulted in a bradycardic period lasting 30-60 min followed by the development of tachycardia. A possible explanation of this effect may be due to the systemic administration of KA. Activation of glutamate receptors in the nucleus ambiguus and the NTS have been found to mediate bradycardia. KA may act on these lower brainstem regions first resulting in a rapid drop in HR. As KA (or the electrical signal) spreads to activate other brain regions such as the hypothalamus, periaqueductal gray matter, caudate putamen complex and cortex, tachycardia is induced. The IML has been reported to produce a weak increase in HR following administration of KA. It is possible that this effect is not significant compared to the bradycardia produced by the nucleus ambiguus and NTS. It could also be proposed that a high dose of systemic KA results in a potent and prolonged activation of glutamate receptors in the IML which could lead to desensitisation, endocytosis or a depolarising block of the receptors (Figure 4.5).

In the present study, females exhibited more pronounced seizure activity and bradycardia than males. This may be due to oestrogen’s effect on different autonomic function. Females have higher levels of circulating oestrogen which may act on the IML, nucleus ambiguus and NTS to enhance vagal tone. Administration of KA to females may act to stimulate the parasympathetic nervous system leading to bradycardia and alterations in the ECG such as P wave lengthening. Males have enhanced sympathetic tone which explains why there is less seizure induced bradycardia and QT interval propagation observed in this experiment. Future experiments using pharmacological intervention will be useful for determining if sex hormones are beneficial for seizure induced cardiac and neuronal damage. Administration of specific
oestrogen or androgen receptor agonists may be an effective prophylactic treatment in epileptic patients at risk of developing SUDEP. It would be interesting to find out if oestrogen has long term beneficial effects or if testosterone is effective at reducing seizure induction. This experiment provides insight into seizure induced arrhythmias and cardiac pathology, however further understanding of sex hormone involvement in the ANS and cardiac glutamate receptors is required.

Figure 4.5: Possible explanation of the effect of KA induced heart rate changes. Blue represents the sympathetic pathways mediated by glutamate transmission. Green represents the parasympathetic pathways mediated by glutamate transmission. Administration of KA activates lower brain regions first resulting in bradycardia. One hour post KA tachycardia and generalised seizures occur as KA activates higher or generalised brain regions. Oestrogen (pink arrows) alters the activity of lower brainstem regions enhancing the effect of KA which may explain why bradycardia is more potent in females. Brain adapted from Iverson et al. (2000)
APPENDIX

5.1 ECG Analysis using LabChart software from AD Instruments

The section of the ECG trace which was to be analysed was highlighted, before opening the ECG Settings. The settings were set up as shown in Figure 5.1. A rat ECG trace was selected with 10 heart beats averaged and the QT interval corrected using the Michell et al. method.

![ECG Settings](image)

**Figure 5.1: ECG settings used in this experiment**

The ECG beat classifier was used to reject heart beats which were distorted by noise, such as during wet dog shakes (Figure 5.2). Large voltages in activity or isoelectric noise indicated a distorted signal ECG trace, this was observed by high frequency spike which no distinguishable ECG pattern. The second box was used to determine the presence of abnormally prolonged RR intervals. This could be examined to determine if there was a skipped heart beat occurred or if the R wave had not be detected. The parameter limits were set as follows: Activity= 10 mV; Isoelectric noise 0.1 mV; RR interval= 0.2 sec; Form factor= 50.
Figure 5.2: ECG beat classifier settings

Figure 5.3 shows the averaging view, the lines were moved if needed to put them in the correct position so that the distance between waves could be determined. The amplitude and the intervals were then presented in the ECG analysis table. This average of the data was used for statistical analysis of ECG variables.

Figure 6.3: ECG averaging view
REFERENCE LIST


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