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# Cardiac electrographic and morphological changes following status epilepticus: Effect of clonidine

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ABSTRACT

*Purpose:* Status epilepticus has been increasingly associated with cardiac injury in both clinical and animal studies. Our group has previously shown that excitotoxic seizure induction results in the formation of ischaemic myocardial infarcts and loss of cardiac haemodynamic function. We hypothesised that attenuation of cardiac sympathetic/parasympathetic balance with a central presynaptic $\alpha_2$ agonist, clonidine, can reduce the development of interictal ECG and ventricular morphological changes resulting from kainic acid (KA; 10 mg/kg) induced status epilepticus in a conscious rat model.

*Methods:* Using simultaneous ECG and electrocorticogram (ECoG) radiotelemetry, animals were randomised into saline controls, saline-pretreated KA and clonidine (100 μg/kg, b.i.d.)-pretreated KA groups. Baseline ECG, ECoG and behavioural score recordings were acquired in conscious animals for 2 h post-KA administration.

*Results:* Bradycardia and low level seizure activity occurred immediately following KA administration. As seizure activity (ECoG spiking and high level seizure behavioural scoring) progressively increased, tachycardia developed. Both QTc prolongation and T wave amplitude were transiently but significantly increased. Clonidine treatment attenuated seizure activity, increased the latency to onset of seizure behaviour and reduced seizure-induced changes in heart rate, QTc interval, and T wave amplitude. Histological examination of the ventricular myocardium revealed hypercontraction band necrosis, inflammatory cell infiltration, and oedema at 48 h post-KA. In contrast, clonidine-treatment in seizure animals preserved tissue integrity and structure.

*Conclusion:* These results demonstrate that KA-induced seizures are associated with altered ECG activity and cardiac structural pathology. We suggest that pharmacological modulation of sympathetic/parasympathetic activity in status epilepticus provides a promising therapeutic approach to reduce seizure-induced cardiomyopathy.



## 1. Introduction

Status epilepticus (SE) is a neurological emergency associated with high mortality (10–30%) occurring predominately within 30 days of the initial convulsive activity.[1] SE is loosely defined as a single or multiple recurring seizures lasting a minimum of 30 min, although there is now a general consensus that seizure duration exceeding 5–10 min should be treated as SE.[2] Previous studies have shown that impaired autonomic regulation and seizure-induced cardiorespiratory alterations can occur during epileptic discharge, leading to cardiac arrhythmias, autonomic imbalance and hypoxia.[3] Symptoms of increased sympathetic activation are frequently observed during seizures, with tachycardia reported in a majority of clinical presentations.[3–5] Electrocardiogram (ECG) wave abnormalities have been reported in 35% of seizures and include atrial fibrillation, ventricular premature depolarisation, QT prolongation and atrioventricular block.[6,7] Most changes are benign, however potentially serious changes occur in 6–13% of seizures.[5,6] Death following SE may result as a consequence of uncontrolled tachycardia leading to malignant ventricular tachyarrhythmia's and long-term myocardial damage.[3] Ictal bradycardia (<40 b.p.m.) is rarely reported, occurring in 2% of seizures but should not be dismissed as a potential contributor to sudden cardiac death.[6,7]

Chemoconvulsant agents have been used to reproduce many of the features of acquired human epilepsy in rodent models.[8]

*Abbreviations:* AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; b.p.m., beats per minute; ECoG, electrocorticogram; H&E, haematoxylin-eosin; HR, heart rate; KA, kainic acid; MSB, Martius scarlet blue; sc, sub-cutaneous; SE, status epilepticus; WDS, wet dog shakes.

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Systemic administration of muscarinic agonists such as pilocarpine, or excitotoxins such as kainic acid (KA) produces SE and neuropathology.[9,10] By binding to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and KA receptors in the brain, KA and domoic acid can initiate persistent hippocampal hyperexcitability which may spread to other brain regions.[11] Excitotoxic seizure induction with KA in rat models produces escalating levels of behavioural responses which include head tremors, wet dog shakes (WDS) and eventually clonic–tonic convulsions.[12,13] Previous studies by our group demonstrated that neither KA nor domoic acid produce direct toxic effects on rat cardiomyocyte or isolated heart preparations.[14] Furthermore, we have shown that domoic acid-induced SE produces identical patterns of structural cardiomyopathy, irrespective of whether the excitotoxin is delivered through central or systemic routes.[14]

Current interventions for the management of SE include the use of anti-epileptic medications with benzodiazepines as a first-line measure and phenytoin, phenobarbital and valproate reserved for unresolved SE.[15,16] SE however can remain refractory in many cases, with 13% indicated to remain uncontrolled even after a third-line intervention.[17] Not surprisingly, sudden cardiac death has been associated with prolonged or drug-refractory SE.[1] In this study, we examined the hypothesis that the cardiomyopathy consequent to the development of SE, occurs at least in part, from a centrally-evoked activation of cardiac sympathetic nerves and that the cardiac structural damage and dysfunction arising may be mitigated by prophylactic treatment with adrenergic modulators. Clonidine, a pre-synaptic $\alpha_2$ adrenoreceptor agonist, was utilised for its ability to centrally reduce noradrenaline/adrenaline release whilst increasing vagal activity, thereby simultaneously producing sympatholytic and parasympathomimetic effects.[18,19] The results of this study provide new insight into the ECG changes which occur during a seizure and suggest potential new therapeutic approaches to reduce the ensuing cardiac damage.

## 2. Materials and methods

### 2.1. Materials

All reagents were purchased from BDH (Palmerston North, New Zealand) and Sigma–Aldrich (Auckland, New Zealand). KA (Tocris, Bristol, UK) and clonidine (Sapphire Bioscience PTY (New South Wales, Australia) were dissolved in normal saline. Surgical materials and drugs were obtained from the University of Otago Animal Welfare Office.

### 2.2. Animals

Sprague–Dawley rats (20 males; 320–350 g) were obtained from the University of Otago Animal Resource Unit. The animals were housed on a 12 h light/dark cycle at 22 °C with food and water *ad libitum*. Experiments were performed in accordance with the University of Otago's Committee on Ethics in the Care and Use of Laboratory Animals and the "Use of Laboratory Animals" (NIH Publication No. 85-23, 1996).

### 2.3. Experimental protocol

Combined ECG and ECoG recordings were obtained in conscious animals through the use of implantable radiotelemeters (Telemetry Research, Auckland, New Zealand) to avoid any confounding effects of anaesthetics on cardiovascular responses.[9,20] Following implantation of the radiotelemeters, animals were randomly allocated into saline-control (no seizure induction, $n = 5$), saline-KA (saline-pretreated, $n = 10$) or clonidine-KA (clonidine-pretreated, $n = 5$) treatment groups. Saline or clonidine (100 μg/kg, sc) were administered twice daily, three days prior to KA challenge and for the duration of the experiment.[19] No animals died prematurely following any drug treatments.

### 2.4. Surgical implantation of telemetric transmitters

Animals were administered Strepcin (benzylpenicillin and dihydrostreptomycin, 250 IU) and carprofen (5 mg/kg, sc) prior to surgery and once every 24 h post-operatively for 3 days. Anaesthesia was elicited using ketamine (75 mg/kg, sc), domitor (medetomidine hydrochloride; 0.5 mg/kg, sc) and atropine (0.05 mg/kg, sc). Body temperature was maintained at 37 °C throughout the surgery. Transmitter implantation and electrode positioning procedures were performed as previously described.[13,21] Animals were housed individually post-surgery and left to recovery for 7 days before seizure induction.

### 2.5. Seizure induction and telemetric/behavioural recordings

The behavioural study was performed in a custom-made perspex observation chamber (1 m × 50 cm × 50 cm). Each rat was left to acclimatise in the chamber prior to study initiation. ECoG and ECG were sampled at 2000 Hz, with receiver filters set to 0.1 Hz high pass and 1000 Hz low pass using a Powerlab 2/25 signal conditioner and LabChart v.6 Pro software (AD Instruments, Sydney, Australia). ECoG, ECG and behavioural data were simultaneously recorded during a 20 min baseline period and for 2 h post-saline or KA administration. Seizures were induced by a single injection of KA (10 mg/kg, sc, maximum volume of 0.35 mL). The control animals were treated with an equivalent volume of saline subcutaneously. Following treatment, the rat was immediately returned to the chamber for behavioural observation. Behaviour was recorded every 15 s for 2 h with discrete changes in behavioural state additionally reported as they occurred. Behaviours were recorded by three different pre-trained observers using an agreed 5-point scale as previously described.[12,13] Normal behaviours (Level 0), discomfort behaviours (Level 1), seizure behaviours confined to the head (Level 2), seizure behaviours associated with limbs or trunk such as wet dog shakes (WDS; Level 3), generalised seizure behaviours (Level 4) and clonic–tonic convulsion (Level 5). The cumulative score was determined as the sum of the maximum score every 15 s over the 2 h recording period. Behavioural seizures could not be conducted in a blinded manner and is a limitation of this study.

### 2.6. ECG and ECoG analysis

Recorded data were post-analysed blindly using pre-established/fixed algorithms within Lab Chart 6 Pro Software. ECG data was analysed using the ECG Analysis module software in order to assess heart rate (HR), QT intervals and T wave amplitudes. Data were analysed every five minutes in one minute blocks over the 2 h observation period. The corrected QT (QTc) was calculated in order to adjust for rate by applying the Mitchell algorithm to the QT interval recordings, $QTc = QT_0/(RR/100)^{1/2}$.[22] This algorithm is designed to correct for the higher HR and altered QRS-T wave morphology in rodents. High amplitude ECoG spiking was defined as sharp electrographic events three-times baseline voltage and counted using LabChart Spike Analysis over a 15 min bin. Movement artefacts were identified on the ECG and eliminated from the ECoG analysis.

### 2.7. Morphological characterisation of myocardial injury

48 h following KA or saline administration the animal was anaesthetised with halothane and the heart was excised. Hearts