Evaluation of a technique to measure heart rate variability in anaesthetised cats

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ABSTRACT

Analysis of heart rate (HR) and heart rate variability (HRV) are powerful tools to investigate cardiac diseases, but current methods, including 24-h Holter monitoring, can be cumbersome and may be compromised by movement artefact. A commercially available data capture and analysis system was used in anaesthetised healthy cats to measure HR and HRV during pharmacological manipulation of HR. Seven healthy cats were subjected to a randomised crossover study design with a 7 day washout period between two treatment groups, placebo and atenolol (1 mg/kg, IV), with the efficacy of atenolol to inhibit β_1 adrenoreceptors challenged by epinephrine. Statistical significance for the epinephrine challenge was set at $P < 0.0027$ (Holm–Bonferroni correction), whereas a level of significance of $P < 0.05$ was set for other variables.

Analysis of the continuous electrocardiography (ECG) recordings showed that epinephrine challenge increased HR in the placebo group ($P = 0.0003$) but not in the atenolol group. The change in HR was greater in the placebo group than in the atenolol group ($P = 0.0004$). Therefore, compared to cats pre-treated with placebo, pre-treatment with atenolol significantly antagonised the tachycardia while not significantly affecting HRV. The increased HR in the placebo group following epinephrine challenge was consistent with a shift of the sympathovagal balance towards a predominantly sympathetic tone. However, the small (but not significant at the critical value) decrease in the normalised high-frequency component (HF_{norm}) in both groups of cats suggested that epinephrine induced a parasympathetic withdrawal in addition to sympathetic enhancement (increased normalised low frequency component or LF_{norm}). In conclusion, this model is a highly sensitive and repeatable model to investigate HRV in anaesthetised cats that would be useful in the laboratory setting for short-term investigation of cardiovascular disease and subtle responses to pharmacological agents in this species.

Introduction

Analysis of heart rate variability (HRV) is a useful non-invasive marker for evaluation of heart rate (HR) autonomic modulation (Lewis and Short, 2010). The control of beat-to-beat HR is dependent upon the fluctuating balance of the sympathetic and parasympathetic input at the sino-atrial node (Kleiger et al., 1992; Chapleau and Sabharwal, 2011). Analysis of HRV will therefore reflect the dynamic interplay between the multiple physiological mechanisms which regulate the instantaneous HR and R-R intervals (intervals between QRS complexes of normal sinus depolarisation) (Malik et al., 1996; Sztajzel, 2004). In addition, HRV analysis provides a means of monitoring the activity of, and any changes in, the state and integrity of the autonomic nervous system (ANS)

(Kleiger et al., 1992; Chapleau and Sabharwal, 2011) and is an important prognostic indicator in cardiovascular disease in human patients (Sztajzel, 2004).

Quantification of HRV often relies on the application of continuous ambulatory electrocardiography (ECG), also termed 24-h Holter ECG (Malik et al., 1996; Goodwin, 1998). In humans, the 24-h Holter ECG is the gold standard diagnostic tool for assessment of arrhythmias and the effectiveness of anti-arrhythmic therapy, and is also utilised to monitor ischaemic S-T segment changes and other parameters during normal daily activity (Maron et al., 1981; Kennedy, 2006). Similarly, the 24-h Holter ECG is widely used by veterinary cardiologists (Goodwin, 1998; Petrie, 2005) and has proven more sensitive than resting ECG for identification and quantification of arrhythmias associated in dogs (O'Sullivan et al., 2008).

It should be understood that HR changes frequently in healthy animals (and humans) in response to a range of physiological stimuli, including breathing, body posture, wake or sleep cycle, move-

ment and anything causing excitement, fear anxiety or pain. Furthermore, many anaesthetic agents and sedatives may alter the function of the ANS by altering blood pressure, baroreflex sensitivity and central nervous system (CNS) function. The application of 24-h Holter ECG has been validated in cats, but analysis with the commercially available software is expensive and requires specific training for interpretation (Ware, 1999; Abbott, 2005; Hanas et al., 2009). However, cats may be more responsive to changes in the environment than dogs, which can make the assessment of a therapeutic intervention on cardiovascular parameters difficult, particularly if only subtle changes are expected (Alipov et al., 2005). Importantly, the effect on HRV changes due to physiological events (Campen et al., 2005), the influence of toxicological substances (Fairchild et al., 2009) or the short term effects of pharmacological agents (Joaquim et al., 2004) on the ANS control of the heart may be more accurately measured in anaesthetised laboratory animals, where movement artefact and environmental stressors are eliminated.

The aim of the current study was to evaluate a technique using a standard laboratory recording system to monitor HR and HRV in anaesthetised cats. The sensitivity of the technique was tested by administering a β_1 adrenoreceptor (β_1-AR) antagonist (atenolol) and a β_1-AR agonist (epinephrine) to the cats to determine if subtle and/or short-lived changes in HR and HRV can be detected. This laboratory-based model could be useful to investigate other potential therapeutic approaches to cardiovascular disease in cats, particularly if responses are expected to be subtle and/or affected by any stress-related increase in sympathetic activity.

Materials and methods

Animals

Cats were recruited from a group of resident teaching population that belongs to the Clinical Studies Centre at the School of Veterinary Science, at the University of Queensland. Suitability of cats for study inclusion was based on assessment of: (1) normal physical examination (including cardiac auscultation); (2) blood haematology, biochemistry, total thyroxine (T4) and urinalysis profile within reference range; (3) indirect Doppler blood pressure measurement (<150 mmHg; Brown et al., 2007), and (4) normal echocardiographic examination. Seven clinically healthy, neutered cats (four males, three females) having a mean ± SD (range) bodyweight (BW) of 4.1 ± 1.1 (6.0–3.0) kg, and age of 1.5 ± 0.9 (approximately 1–3) years were recruited for the study. All cats (six Domestic shorthairs and one Domestic longhair) were kept in group housing, fed ad libitum and maintained in an animal facility with enrichment activities. The study was approved from the University of Queensland Animal Ethics Committee (AEC approval number SVS/040/09).

Echocardiography

Echocardiographic examination was performed following subcutaneous (SC) administration of 0.1 mg/kg hydromorphone hydrochloride (Dilaudid) and 0.1 mg/kg acepromazine (ACP 2) (Campbell and Kittleson, 2007). Each cat was manually restrained on right lateral recumbency. Routine two-dimension images (Esoate MyLab30 Gold) were obtained using a 12 MHz probe placed on the right precordium (Paige et al., 2009) by one author (AR). The echocardiograms were recorded and analysed as previously described (Thomas et al., 1993). Inclusion of healthy cats with a normal heart were based on the following: (1) the left ventricular free wall end-diastolic diameter (LVFWDd) and the inter-ventricular septal end-diastolic diameter (IVSd) were <5.5 mm; (2) the left atrium to aortic diameter ratio was <1.5; (3) there were no other heart structural abnormalities (Kittleson and Kienle, 1998).

Anaesthesia

Food was withheld for 12 h before induction of anaesthesia and water was made available prior to administration of pre-anaesthetic medication. All cats were given the same pre-medication protocol as used for ECG examination. Approximately 20 min later, each cat was induced with 5 mg/kg alfaxalone intravenously (IV, Alfaxan) (Zaki et al., 2009; Taboada and Murison, 2010), incubated and maintained on ~1.5% isoflurane (Delvet) delivered in 100% oxygen. During anaesthesia, the cats' hydration was maintained with 0.9% normal saline (2.5 mL/kg/h, IV) and body temperature was maintained using heated water bottles. The respiratory rate, pulse rate and haemoglobin oxygen saturation (using handheld pulse oximeter) were also monitored.

Study design

Each cat was subjected to a randomised crossover study design separated by a 7 day washout period (>25 atenolol elimination half-lives) between treatments (Fig. 1). Cats were first anaesthetised as described above. To minimise data variation, cats were randomly divided into two groups: Group 1 treated with placebo (week 1) and atenolol (week 2) and Group 2 treated with atenolol (week 1) and placebo (week 2). All cats were subjected to the same treatment at the end of the study.

Post-placebo or post-atenolol challenge

The HR was allowed to stabilise for 15 min before a 30 min baseline HR measurement was recorded (Fig. 1). Each cat was treated with either 1 mg/kg atenolol IV (Tenormin) or placebo (0.9% sodium chloride, IV, in an equal volume to calculated atenolol). The HR was allowed to stabilise for 15 min before the HR measurement was recorded for a further 30 min.

Post-epinephrine challenge

A pilot study was initially conducted to determine the dose of epinephrine for challenge studies to determine how effectively atenolol was inhibiting the β receptor. Three healthy cats pre-treated with atenolol or placebo on two different days, received sequential doses of epinephrine 0.05 μg/kg, 0.5 μg/kg and 5.0 μg/kg, where
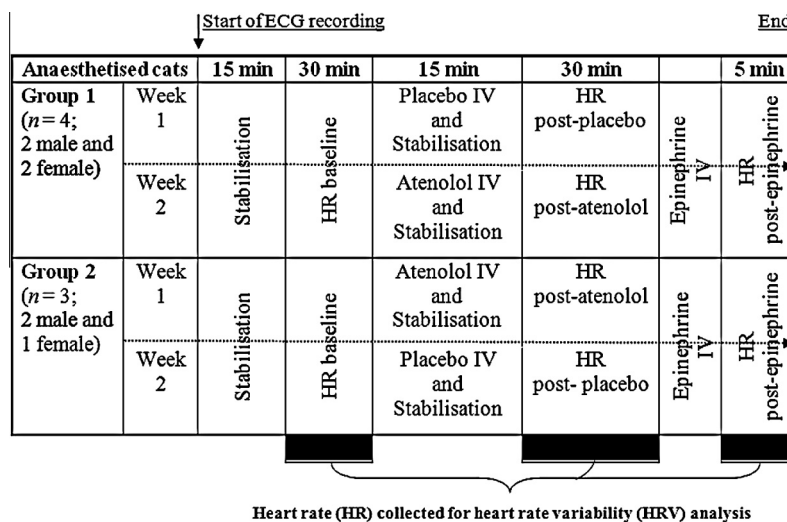

Anaesthetised cats		15 min	30 min	15 min	30 min		5 min
Group 1 (*n* = 4; 2 male and 2 female)	Week 1	Stabilisation	HR baseline	Placebo IV and Stabilisation	HR post-placebo	Epinephrine IV	HR post-epinephrine
	Week 2			Atenolol IV and Stabilisation	HR post-atenolol		
Group 2 (*n* = 3; 2 male and 1 female)	Week 1	Stabilisation	HR baseline	Atenolol IV and Stabilisation	HR post-atenolol	Epinephrine IV	HR post-epinephrine
	Week 2			Placebo IV and Stabilisation	HR post-placebo		

Heart rate (HR) collected for heart rate variability (HRV) analysis

Fig. 1. Timeline of the experimental study and the respective time point of data collected for analysis.