# Chronic atrial tachypacing induced atrial fibrillation in conscious dogs

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## Introduction

Atrial fibrillation (AF) is the most frequent sustained arrhythmia that is a major cause of morbidity and mortality mainly due to strike and heart failure [Kannel *et al*., 1982]. AF represents a large and still growing public health burden, its prevalence is 0.5% in patients in their 50s but it is almost 10% over the age of 80 [Benjamin *et al*., 1998]. Current state-of-the-art invasive electrophysiological intervention and pharmacological therapy of AF have limited efficacy, especially in patients with remodeled atria and persistent/permanent AF. Traditional antiarrhythmic drugs used for atrial fibrillation treatment were developed for ventricular arrhythmias, consequently, they exhibit ventricular electrophysiological side effects. Therefore, there is a clearly unmet need for the development of better pharmacological management of AF [Dobrev *et al*., 2012]. It is essential to have adequate animal experimental methods closely resembling human AF for the development and *in vivo* testing of novel compounds. In the Department of Pharmacology and Pharmacotherapy, University of Szeged, an *in vivo* model has been established, to test the efficacy of novel compounds on AF in unrestrained conscious dogs. The conscious animal method has the advantage of testing developmental compounds in a setting that is not influenced by the cardiac electrophysiological effects of anaesthetic agents. The method relies on chronic rapid atrial pacing to induce electrical and structural remodeling of the atria [Franz *et al*., 1997.].

## Methods

### *Ethical approval*

Animal experimental ethical approvals are necessary for the surgery and experiments described in this document. All experiments are carried out in compliance with the Guide for the Care and Use of Laboratory Animals (USA NIH publication No 85–23, revised 1996) and conform to the Directive 2010/63/EU of the European Parliament. The protocols have been approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval number: I-74-5-2012), and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development, Hungary (approval number: XIII/1211/2012).

### *Animals, surgery, treatment and measured parameters*

Adult male Beagle dogs (14–16 kg) are anesthetized with ketamine (induction: 10 mg/kg, maintenance: 2 mg/kg every 20 min) and xylazine (induction: 1 mg/kg, maintenance: 0.2 mg/kg every 20 min) and are mechanically ventilated. Following the implantation of two bipolar pacemaker electrodes (Logos, Karios; Biotronik Hungaria) into the right atrial appendage and apex

of the right ventricle, the electrodes are connected to pacemakers in subcutaneous pockets in the neck area, radiofrequency catheter ablation of the AV node was performed to avoid high atrial pacing rates propagating into the ventricles. The ventricular pacemaker is set between 80 to 90 beats/min, following the baseline heart rate of the dog before the operation. Following recovery from surgery (3 days), high frequency right atrial pacing is started at 400 beats/min, maintained for 6 to 7 weeks before the experiments to allow electrical remodeling of the atria (monitored by the measurement of the right atrial effective refractory period (AERP) every second day). ECGs are digitally recorded and stored and later evaluated off-line using SPEL Advanced Haemosys software (v3.2, Experimetria Ltd., Budapest, Hungary). The AERPs are measured at basic cycle lengths (BCL) of 150 and 300 ms with a train of 10 stimuli (S1) followed by an extrastimulus (S2), with the AERP defined as the longest S1S2 interval that did not produce a response. AERP shorter than 80 ms cannot be measured in conscious animals (pacemaker measurement limit). On the day of the experiment atrial pacing is stopped, continuous recording of the electrocardiogram commences using precordial leads and the AERP is measured. A control set (25 times) of 10-second long rapid atrial bursts (800 beats/min, at twice threshold) are performed in order to induce atrial fibrillation in conscious dogs preceded by a bolus infusion of vehicle. Vehicle typically can be 20 mL of a mixture of DMSO + β-hydroxypropyl-cyclodextrin + saline (all from Sigma, St. Louis, USA), with DMSO concentration less than 0.1%, infused in 15 min, in case of compounds with poor solubility. During the control 25 bursts and subsequent AF episodes, a continuous infusion of vehicle is maintained (in a volume of 1.7 mL/kg/min). Following the measurement of AERP, the investigational compound is infused in the required dose (in 15 min bolus + maintenance) and AF is again induced 25 times. An identical procedure is repeated with another, higher dose on the same dog if required. The incidence of AF, the total duration of AF, the average duration of AF episodes are measured along with changes in atrial refractory period and QT interval. QT intervals are measured before the 12th burst and are not corrected for heart rate since QT measurements are made at heart rate set to 80. However, if required, the ventricular pacemaker can be set to different rates to measure QT at different heart rates, in this case, the QTc is also calculated using a formula developed for Beagle dogs as follows: QTc = QT – (0,087 * (RR-1000) [Tattersal *et al*., 2006]. Representative ECG recordings illustrating the model are shown on Figures 1 and 2.

***Treatment possibilities***

The department is prepared to administer the test compounds acutely or chronically, depending on the need of the client. In case of acute administration, oral or different routes of parenteral administrations are all options. Intravenous administration is carried out by programmable infusion

pumps (Terufusion TE-3, Terumo Europe, Leuven, Belgium). Chronic treatment is carried out orally with pre-made capsules in the required dose (mg/bwkg) every day at the given time point for the set time period (weeks, months). The body weight of animals are continuously monitored to adjust the amount of compound administered.

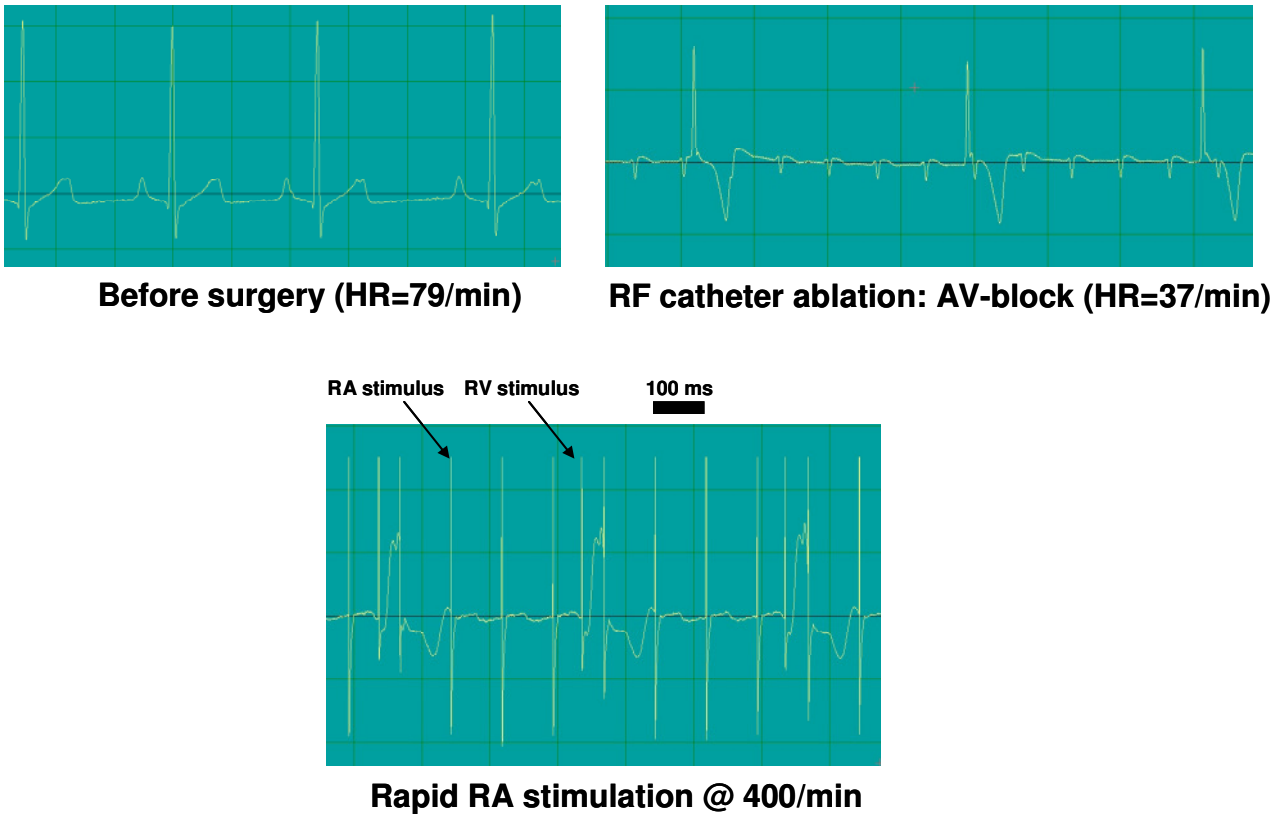


**Figure 1.** Representative ECG recordings from different stages of the procedure of chronically instrumented conscious Beagle dogs (before surgery, following AV node ablation leaging to third degree AV block, and during 400/min right atrial tachypacing). HR=heart rate; RF=radiofrequency ; RA= right atrial; RV=right ventricular

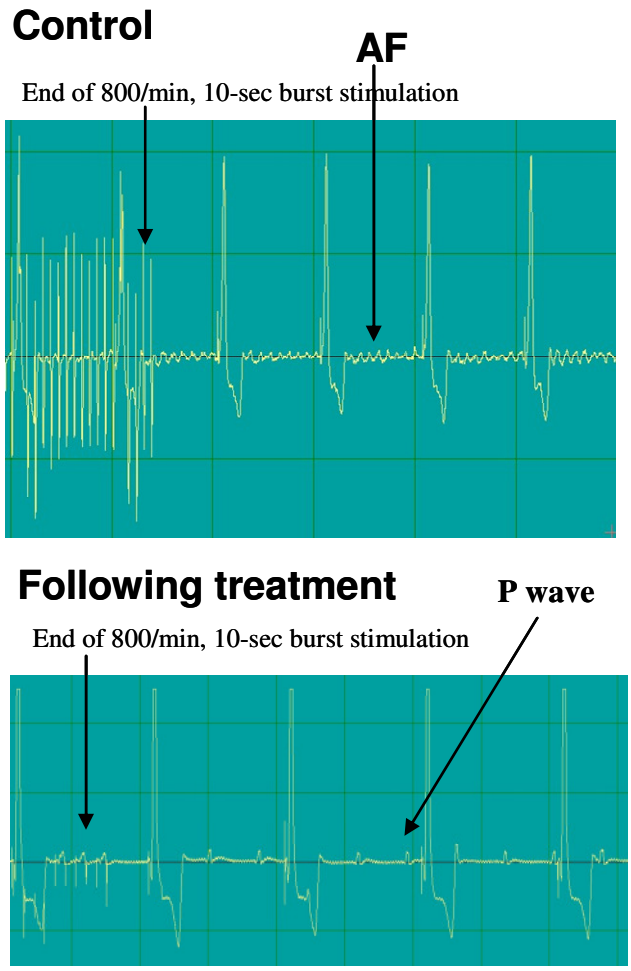


**Figure 2.** Representative ECG recordings showing 800/min burst provocation of AF in conscious dogs before (control) and following treatment with an effective compound. AF=atrial fibrillation

*Plasma and tissue samples*

In acute experiments, blood samples are taken imediately before and at set time points during the experiments following the administration of test compounds for the determination of plasma sample test drug levels. In chronic experiments, blood samples are taken at required time points before and following commencement of administration of the test drug. Centrifuged plasma is stored at -20$^{o}$C for further plasma level determination. For tissue sample work, animals are sacrificed following sedation and terminal anaesthesia (in compliance with current animal experimental ethical regulations). Following thoracotomy, the hearts are quickly removed and are placed into ice cold cardioplegic solution, then tissue samples are taken from different regions of the heart. Also, if required by the client, for further *in vitro* experiments atrial and ventricular tissue preparations are taken for action potential and isolated cardiomyocyte ionic current (patch-clamp) measurements. In case it is required by the client, tissue samples can be frozen in liquid nitrogen, stored in 2 ml

cryotubes and later analyzed for required protein and mRNA etc. measurements with our molecular biological setups.

The described model is available in our Department for clients wishing to test the *in vivo* efficacy of novel compounds in development for the treatment of atrial fibrillation.

**References**

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