ARTICLE

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Comparison of the effects of $I_{K,ACh}$, I_{Kr}, and I_{Na} block in conscious dogs with atrial fibrillation and on action potentials in remodeled atrial trabeculae

Viktor Juhász, Tibor Hornyik, Attila Benák, Norbert Nagy, Zoltán Husti, Róbert Pap, László Sághy, László Virág, András Varró, and István Baczkó

Abstract: Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and a major cause of morbidity and mortality. Traditional antiarrhythmic agents used for restoration of sinus rhythm have limited efficacy in long-term AF and they may possess ventricular proarrhythmic adverse effects, especially in patients with structural heart disease. The acetylcholine receptor-activated potassium channel ($I_{K,ACh}$) represents an atrial selective target for future AF management. We investigated the effects of the $I_{K,ACh}$ blocker tertiapin-Q (TQ), a derivative of the honeybee toxin tertiapin, on chronic atrial tachypacing-induced AF in conscious dogs, without the influence of anesthetics that modulate a number of cardiac ion channels. Action potentials (APs) were recorded from right atrial trabeculae isolated from dogs with AF. TQ significantly and dose-dependently reduced AF incidence and AF episode duration, prolonged atrial effective refractory period, and prolonged AP duration. The reference drugs propafenone and dofetilide, both used in the clinical management of AF, exerted similar effects against AF in vivo. Dofetilide prolonged atrial AP duration, whereas propafenone increased atrial conduction time. TQ and propafenone did not affect the QT interval, whereas dofetilide prolonged the QT interval. Our results show that inhibition of $I_{K,ACh}$ may represent a novel, atrial-specific target for the management of AF in chronic AF.

Key words: atrial fibrillation, conscious dog, $I_{K,ACh}$, propafenone, tertiapin-Q.

Résumé : La fibrillation auriculaire (FA) constitue l'arythmie cardiaque soutenue la plus courante et une cause majeure de morbidité et de mortalité. Les antiarythmiques traditionnels utilisés pour rétablir le rythme sinusal ont une efficacité limitée dans la FA à long terme, et ils pourraient être associés à des effets indésirables favorisant les arythmies ventriculaires, particulièrement chez les patients présentant une maladie cardiaque structurelle. Le canal potassique activé par le récepteur de l'acétylcholine ($I_{K,ACh}$) représente une cible auriculaire sélective pour la prise en charge future de la FA. Nous avons étudié les effets de la tertiapine-Q (TQ), un bloqueur du canal $I_{K,ACh}$ dérivé de la tertiapine (toxine de l'abeille), sur la stimulation auriculaire rapide chronique provoquée par la FA chez des chiens conscients, sans l'influence d'anesthésiques, qui peuvent moduler un certain nombre de canaux ioniques cardiaques. Nous avons enregistré des potentiels d'action (PA) à partir de trabécules d'oreillettes droites isolées de chiens présentant une FA. De façon marquée et proportionnelle à la dose, la TQ permettait de réduire la fréquence et la durée des épisodes de FA, ainsi que de prolonger la période réfractaire effective et la durée du PA dans l'oreillette. La propafénone et le dofétilide, deux médicaments de référence utilisés dans la prise en charge clinique de la FA, exerçaient des effets similaires contre la FA in vivo. Le dofétilide entraînait une prolongation de la durée du PA, alors que la propafénone entraînait une augmentation des temps de conduction dans l'oreillette. Le dofétilide entraînait une prolongation de l'intervalle QT, alors que la TQ et la propafénone n'avaient aucun effet sur ce paramètre. Nos résultats montrent que l'inhibition du canal $I_{K,ACh}$ pourrait représenter une nouvelle cible propre à l'oreillette pour la prise en charge de la FA chronique. [Traduit par la Rédaction]

Mots-clés : fibrillation auriculaire, chien conscient, $I_{K,ACh}$, propafénone, tertiapine-Q.

Introduction

Atrial fibrillation (AF) is the most prevalent chronic arrhythmia (Kannel et al. 1982), associated with increased morbidity and mortality due to thromboembolic complications and concomitant heart failure (Wolf et al. 1991; McManus et al. 2012). Its incidence and prevalence is rapidly increasing with the aging of the population and the number of cases will probably double by 2060 in the European Union (Krijthe et al. 2013). There is a great unmet need for more efficacious and safer pharmacological AF therapy, because drugs currently used for rhythm control (*i*) may significantly increase the risk for torsades de pointes arrhythmias due to their ventricular electrophysiological effects (Taira et al. 2010); (*ii*) can promote adverse vascular events (Connolly et al. 2011); (*iii*) can exhibit reduced efficacy in persistent AF (Vos et al. 1998); (*iv*) and the most effective antiarrhythmic drug, amiodarone, has serious extracardiac side effects when administered chronically (Vorperian

et al. 1997; Santangeli et al. 2012). In addition, as part of the pathological electrical remodeling that occurs in AF, the expression of a number of ion channels and exchangers is altered (Nattel et al. 2008), which can modify the arrhythmia substrate and increase triggered activity resulting in AF to become self-sustaining (Wijffels et al. 1995), and can significantly alter potential drug targets.

A potential approach to improve pharmacotherapy of AF is the identification of drug targets ideally expressed only in atrial tissue (Ravens et al. 2013), because atrial selective ion channel modulation would be devoid of ventricular proarrhythmic adverse effects. The Kv1.5 channel that conducts the ultrarapidly activating outwardly rectifier I_{Kur} represents such a target (Wang et al. 1993; Gaborit et al. 2007; Ford and Milnes 2008), because its block prolongs the atrial action potential duration (APD) and effective refractory period (ERP) (Amos et al. 1996). However, data regarding changes in I_{Kur} expression are inconsistent in animal experimental models of AF and human studies (Yue et al. 1997; Bosch et al. 1999; Grammer et al. 2000), with some clinical studies showing downregulation of the channel in patients with chronic AF (Van Wagoner et al. 1997). Another target with relative atrial selectivity is the acetylcholine receptor-activated inwardly rectifying $I_{K,ACh}$ (Kir3.1/Kir3.4) (Cha et al. 2006; Ehrlich and Nattel 2009), which is found in larger amounts in the atria than in the ventricles (Krapivinsky et al. 1995). Importantly, in contrast to the fact that $I_{K,ACh}$ downregulation was also observed in chronic AF (Dobrev et al. 2001), a constitutively active component of $I_{K,ACh}$ was detected in patients with persistent AF (Dobrev et al. 2005). Therefore, $I_{K,ACh}$ block represents a promising target for the treatment of AF. The selective inhibition of $I_{K,ACh}$ was shown to exhibit beneficial effects against AF in different animal models anesthetized with a combination of isoflurane and thiopental (Hashimoto et al. 2006; Yamamoto et al. 2014). However, thiopental and isoflurane have well-known ion channel modulating properties. Thiopental blocks $I_{Ca,L}$, I_{K1}, and I_{Ks} (Carnes et al. 1997; Heath and Terrar 1996; Morey et al. 1997; Pancrazio et al. 1993; Sakai et al. 1996) and isoflurane blocks $I_{Ca,L}$ and I_{Ks} (Buljubasic et al. 1992; Suzuki et al. 2003), modulates mitochondrial calcium-activated K$^+$ channels (Kinoshita et al. 2016). These different effects on cardiac ion channels can profoundly influence the outcome of atrial and ventricular arrhythmia studies depending on the anesthetic used, including thiopental and isoflurane (Freeman et al. 1990; Napolitano et al. 1996; Baczkó et al. 1997).

Therefore, the aim of the present study was to evaluate the effect of the selective $I_{K,ACh}$ blocker tertiapin-Q (TQ), a more stable derivative of the honeybee venom toxin tertiapin (Jin and Lu 1999), on atrial ERP and inducibility and duration of AF in a conscious canine model of chronic atrial tachypacing-induced AF, devoid of any possible confounding ion channel modulating effects of intravenous and (or) volatile anesthetics. Also, we compared the effects of TQ to those of propafenone (class IC) and dofetilide (class III), both antiarrhythmic agents that are clinically used for AF rhythm control. Finally, for the first time, cardiac cellular electrophysiological effects of these compounds were compared in atrial trabeculae isolated from dogs with chronic right atrial tachypacing-induced AF using the conventional microelectrode technique.

Materials and methods

Ethical issues
The experiments complied with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication No. 85–23, revised 1996). The protocols had been approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (I-74-5-2012), and by the Department of Animal Health and Food Control of the Ministry of Agriculture (XIII/1211/2012).

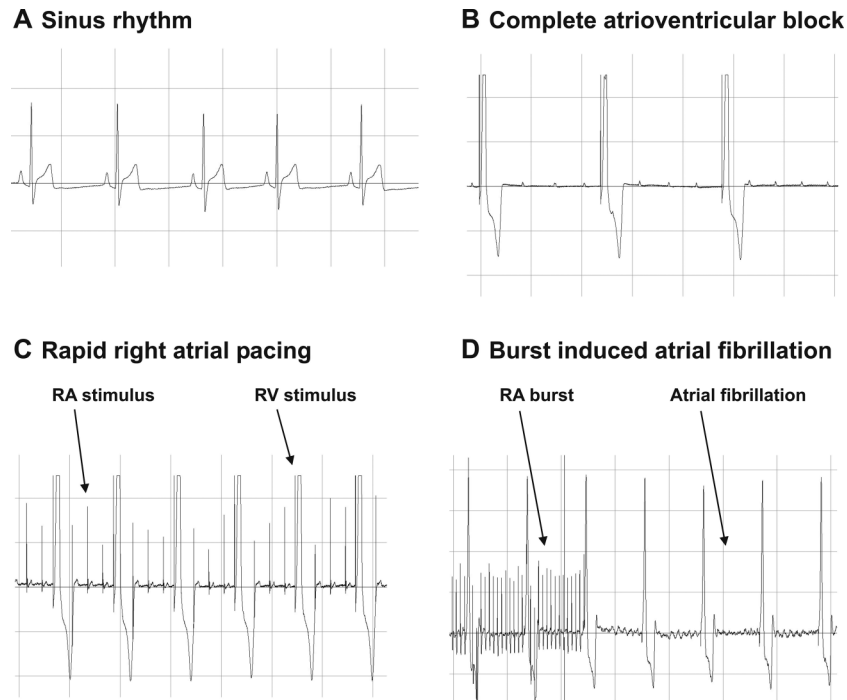### Chronic atrial tachypacing-induced AF in conscious dogs
Male Beagle dogs ($n = 6$) weighing 12–15 kg were used for the experiments. The dogs were accommodated to experimental personnel and equipment, every day for a week before the start of the studies. The pacemaker and pacemaker electrode implantation procedures were performed under ketamine (Richter Gedeon Ltd., Hungary; induction: 10 mg/kg, i.v., maintenance: 2 mg/kg, every 20 min) + xylazine (CP-Pharma Handelsges, Germany; induction: 1 mg/kg, maintenance: 0.2 mg/kg, every 20 min) anesthesia as described previously (Baczkó et al. 2014). Briefly, 2 bipolar pacemaker electrodes (Synox SX 53-JBP and Synox SX 60/15-BP; Biotronik Hungary Ltd., Hungary) were positioned into the right atrial appendage and apex of the right ventricle, respectively, and the electrodes were connected to pacemakers (Logos DS and Philos S; Biotronik Hungary Ltd., Hungary) in subcutaneous pockets in the neck area, followed by radiofrequency catheter ablation of the AV node (Figs. 1A and 1B). The pacemakers were programmed by the ICS 3000 Programmer (Biotronik Hungary Ltd., Hungary). Following recovery from surgery (3–5 days), right atrial tachypacing was started at 400 beats/min (Fig. 1C), maintained for 6–7 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). The AERPs were 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 S1–S2 interval that did not produce a response.

On the day of the experiment, atrial pacing was stopped and continuous recording of the electrocardiogram started using precordial leads and the AERP was measured. A control set (25 times) of 10-second-long rapid atrial bursts (800 beats/min, at twice threshold) were performed to induce AF in conscious dogs (Fig. 1D) preceded by a bolus infusion of vehicle in 15 min. Following the measurement of AERP, additional sets of atrial bursts were applied subsequent to either TQ (Tocris Bioscience, Bristol, UK; 18 µg/kg then 56 µg/kg), or dofetilide (Sigma–Aldrich, 25 µg/kg), or propafenone (Rytmonorm, Mylan EPD Ltd., Hungary; 0.3 mg/kg then 1 mg/kg) i.v. administration. At least 4 days were allowed for washout between in vivo experiments with different compounds. All intravenous infusions were performed using a programmable infusion pump (Terufusion TE-3; Terumo Europe, Leuven, Belgium). The ECG was recorded using precordial leads and was digitized and stored on a computer for off-line analysis using National Instruments data acquisition hardware (National Instruments, Austin, Texas, USA) and SPEL Advanced Haemosys software (version 3.2, MDE Heidelberg GmbH, Heidelberg, Germany). The incidence of AF, the total duration of AF, and the average duration of AF episodes were measured and calculated along with changes in AERP and QT interval. QT intervals were measured on dogs with pacemaker implantation before the 12th burst and were not corrected for heart rate because QT measurements were made at the heart rate set to 80 beats/min by the ventricular pacemaker. Experiments were performed in freely moving conscious dogs so that any effects of anesthetics on AERP and AF could be ruled out.

Action potential recordings from canine right atrial trabeculae with the conventional microelectrode technique
The dogs from the in vivo AF studies were used 4 days following their completion to allow washout of the last applied compound. Following sedation (xylazine, 1 mg/kg, i.v., and ketamine, 10 mg/kg, i.v.) and anesthesia (pentobarbital, Sigma–Aldrich, 30 mg/kg, i.v.), the heart was rapidly removed through right lateral thoracotomy. The hearts were immediately rinsed in oxygenated modified Locke's solution containing (in mmol/L): NaCl 128.3, KCl 4, CaCl$_2$ 1.8, MgCl$_2$ 0.42, NaHCO$_3$ 21.4, and glucose 10. The pH of this solution was set between 7.35 and 7.4 when saturated with the mixture of 95% O$_2$ and 5% CO$_2$ at 37 °C. Isolated right atrial trabeculae were obtained and individually mounted in a tissue chamber with a volume of 50 mL. The preparations were stimulated through a

Fig. 1. Representative ECG recordings from a conscious dog (control) showing (A) sinus rhythm (heart rate = 75/min), (B) complete atrioventricular block following radiofrequency catheter ablation (ventricular rate set to 50/min by ventricular pacemaker), (C) right atrial (RA) tachypacing at 400/min, and (D) right atrial burst pacing (800/min) induced atrial fibrillation. RV, right ventricular.

A Sinus rhythm

B Complete atrioventricular block

C Rapid right atrial pacing

RA stimulus   RV stimulus

D Burst induced atrial fibrillation

RA burst   Atrial fibrillation

pair of platinum electrodes in contact with the preparation using rectangular current pulses of 2 ms duration. The stimuli were delivered at a constant BCL of 500 ms for at least 60 min allowing the preparation to equilibrate before the measurements were initiated. Transmembrane potentials were recorded using conventional glass microelectrodes, filled with 3 mol/L KCl and having tip resistances of 5–20 MΩ, connected to the input of a high impedance electrometer (type 309; MDE Heidelberg GmbH, Heidelberg, Germany) that was coupled to a dual beam oscilloscope. The conduction time, maximum diastolic potential, action potential amplitude, and APD measured at 25%, 50%, and 90% of repolarization (APD$_{25}$, APD$_{50}$, and APD$_{90}$, respectively) were evaluated off-line using a custom made software running on an IBM compatible computer equipped with an ADA 3300 analogue-to-digital data acquisition board (Real Time Devices Inc., State College, Pennsylvania, USA) having a maximum sampling frequency of 40 kHz. Stimulation with a constant BCL of 500 ms was applied during the course of the experiments. We aimed at maintaining the same impalement throughout each experiment; however, in case the impalement became dislodged, adjustment was performed and the experiment continued if AP characteristics of the re-established impalement deviated less than 5% from the previous measurement. Due to the contractility of these preparations, some of the impalements had to be repeated, therefore the maximum upstroke velocity (v_{max}) of the action potentials was not evaluated, and the conduction time was used to assess class I activity in the manuscript.

Compounds

TQ (Tocris Bioscience, Bristol, UK) was dissolved in distilled water for conventional microelectrode experiments (stock solution: 30 μmol/L), and in saline for in vivo experiments. Dofetilide (Sigma–Aldrich) was dissolved in DMSO to obtain a stock solution of 1 mmol/L for microelectrode experiments, and the stock solution was diluted in saline for in vivo experiments. For microelectrode experiments, propafenone (Sigma–Aldrich) was dissolved in DMSO (stock solution: 10 mmol/L), and in in vivo experiments, propafenone was applied using the commercially available 3.5 mg/mL ampule

(Rytmonorm, Mylan EPD Ltd., Hungary). Each stock solution was diluted prior to the actual experiment.

Statistical analysis

All data are expressed as mean ± SEM. Statistical analysis was carried out using ORIGIN 8.1 (Microcal Software, Northampton, Massachusetts, USA). Differences between means were compared by one-way ANOVA followed by Student's t test. Data were considered statistically significant when $p < 0.05$.
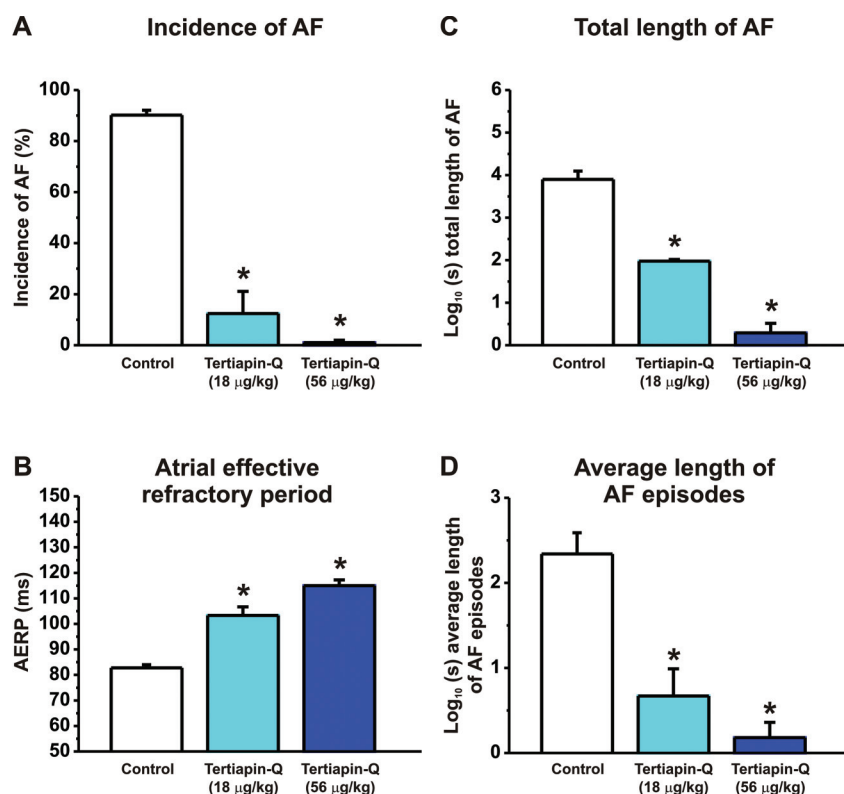
Results

Effects of the $I_{K,ACh}$ blocker TQ, the I_{Kr} blocker dofetilide, and the I_{Na} blocker propafenone on right AERP in conscious dogs

Before the commencement of right atrial tachypacing at 400 beats/min, right AERP was 117 ± 5.8 and 127 ± 6.4 ms in conscious dogs ($n = 6$; at basic cycle lengths of 150 and 300 ms, respectively). Rapid right atrial pacing for 6–7 weeks markedly shortened right AERP, as shown on panel B of Figs. 2, 3, and 4 (measured at the basic cycle length of 300 ms). AERP was significantly and dose-dependently prolonged by TQ at both cycle lengths of 300 ms (Fig. 2B), and of 150 ms: 82.3 ± 1.48 ms in control vs. 93.3 ± 3.33 ms ($n = 6$, $p < 0.05$) following 18 μg/kg and 106.7 ± 2.11 ms ($n = 6$, $p < 0.05$) following 56 μg/kg. The AERP was also significantly prolonged by dofetilide (Fig. 3B; at 150 ms BCL: 81.0 ± 1.81 ms in control vs. 98.3 ± 3.07 ms ($n = 6$, $p < 0.05$) following 25 μg/kg). Only the larger dose of propafenone increased AERP at the BCL of 150 ms: 80.2 ± 0.98 ms in control vs. 85.0 ± 2.89 ms ($n = 6$, $p > 0.05$) following 0.3 mg/kg and 96.7 ± 3.33 ms ($n = 6$, $p < 0.05$) following 1 mg/kg, while the AERP was significantly increased by both propafenone doses at the cycle length of 300 ms (Fig. 4B).

Effects of TQ, dofetilide, and propafenone on burst-induced AF in conscious dogs

Rapid right atrial bursts at 800/min did not induce any AF in any of the animals before the commencement of chronic right atrial

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Fig. 2. Effect of tertiapin-Q (TQ; 18 and 56 μg/kg, i.v.) administration on atrial tachypacing-induced experimental atrial fibrillation (AF) in conscious dogs. The data show that administration of both 18 and 56 μg/kg TQ significantly (A) reduced the incidence of AF, (B) increased the atrial effective refractory period (AERP), and decreased (C) the total duration of AF and (D) the average duration of AF episodes in conscious dogs. $*p < 0.05$; significantly different from control values, one-way ANOVA, $n = 6$ animals. AERP shown in the figure was measured at the basic cycle length of 300 ms. [Colour online.]

tachypacing. Infusion of the $I_{K,ACh}$ blocker TQ dose-dependently and robustly reduced the incidence of right atrial burst-induced AF (Fig. 2A), the total duration of AF (Fig. 2C), and the average duration of AF episodes (Fig. 2D) in conscious dogs. The antiarrhythmic effect of TQ was then compared with the class III compound dofetilide and class IC drug propafenone, both used in clinical settings for rhythm control in AF management. Both dofetilide (Fig. 3A) and propafenone (Fig. 4A) reduced AF incidence, the total duration of AF (Figs. 3C and 4C), and the average duration of AF episodes (Figs. 3D and 4D). These results clearly show that TQ exhibits marked antiarrhythmic effect against AF in conscious dogs, and this effect seemed to be stronger than those of dofetilide and propafenone.

Effects of TQ, dofetilide, and propafenone on the QT interval in conscious dogs

Importantly, none of the investigated doses of TQ prolonged the QT interval in conscious dogs, yielding 283.0 ± 10.36 ms in control vs. 281.8 ± 13.29 ms ($n = 6$, $p > 0.05$) following 18 μg/kg and 268.2 ± 17.75 ms ($n = 6$, $p > 0.05$) following 56 μg/kg. Dofetilide (25 μg/kg) significantly prolonged the QT interval in conscious dogs, from 265.8 ± 8.68 ms in control to 302.8 ± 10.53 ms ($n = 6$, $p < 0.05$). Propafenone did not influence the duration of the QT interval: 268.0 ± 8.41 ms in control vs. 265.4 ± 9.34 ms ($n = 6$, $p > 0.05$) following 0.3 mg/kg and 272.2 ± 7.70 ms ($n = 6$, $p > 0.05$) following 1 mg/kg.
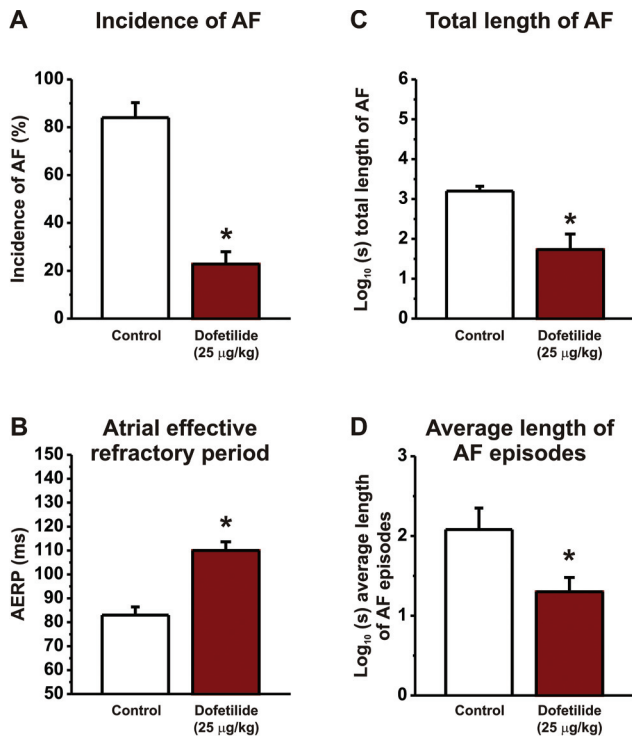
Effects of TQ, dofetilide, and propafenone on action potentials in atrial trabeculae isolated from dogs with AF

To evaluate changes in action potential parameters following chronic atrial tachypacing, in preliminary experiments from non-instrumented dogs in sinus rhythm, the following action potential

parameters were measured from right atrial trabeculae: conduction time, 4.4 ± 0.3 ms; action potential amplitude, 107.1 ± 1.6 mV; diastolic potential, −87.9 ± 1.2 mV; APD_{25}, 28.7 ± 2.0 ms; APD_{50}, 64.5 ± 3.8 ms; APD_{90}, 133.5 ± 5.4 ms ($n = 12$). These APD values in non-instrumented dogs were significantly longer at all investigated percentages of repolarization than those following chronic right atrial tachypacing (see APD values following chronic atrial tachypacing as control in Fig. 5).

Right atrial trabeculae were isolated from the dogs used for the in vivo AF studies, allowing washout of the last compound tested. The effects of TQ (30 nmol/L), dofetilide (100 nmol/L), and propafenone (1 μmol/L) on the action potential configuration and action potential parameters are shown in Fig. 5. All measurements were performed at the cycle length of 500 ms. TQ significantly prolonged the action potential at all percentages of repolarization (APD_{25}, APD_{50}, and APD_{90}) in right atrial trabeculae from dogs with AF (Fig. 5 bottom panel). TQ did not influence conduction time (4.6 ± 0.5 ms in control vs. 4.6 ± 0.3 ms following TQ, $n = 7$, $p > 0.05$), action potential amplitude (107.0 ± 1.4 mV in control vs. 107.0 ± 3.4 mV following TQ, $n = 7$, $p > 0.05$), diastolic potential (−82.9 ± 1.1 mV in control vs. −81.7 ± 1.2 mV following TQ, $n = 7$, $p > 0.05$). Dofetilide significantly prolonged the APD only at 90% of repolarization (Fig. 5 bottom panel). Dofetilide did not alter conduction time (3.7 ± 1.0 ms in control vs. 3.6 ± 0.9 ms following dofetilide, $n = 4$, $p > 0.05$), action potential amplitude (109.8 ± 1.4 mV in control vs. 111.3 ± 0.8 mV following dofetilide, $n = 4$, $p > 0.05$), diastolic potential (−87.5 ± 3.4 mV in control vs. −88.3 ± 2.8 mV following dofetilide, $n = 4$, $p > 0.05$). Propafenone did not prolong the atrial action potential (Fig. 5 bottom panel), did not influence the action potential amplitude (101.4 ± 2.7 mV in control vs. 101.8 ± 2.2 mV following propafenone, $n = 5$, $p > 0.05$) or diastolic potential (−83.1 ± 0.5 mV in

Fig. 3. Effect of the administration of the class III antiarrhythmic dofetilide (25 µg/kg, i.v.) on atrial tachypacing-induced experimental atrial fibrillation (AF) in conscious dogs. The data show that dofetilide significantly (A) reduced the incidence of AF, (B) prolonged the atrial effective refractory period (AERP), and decreased (C) the total duration of AF and (D) the average duration of AF episodes in conscious dogs. $*p < 0.05$; significantly different from control values, one-way ANOVA, $n = 6$ animals. AERP shown in the figure was measured at the basic cycle length of 300 ms. [Colour online.]

control vs. −84.0 ± 1.5 mV following propafenone, $n = 5$, $p > 0.05$), but significantly increased conduction time (3.1 ± 0.3 ms in control vs. 3.9 ± 0.3 ms following propafenone, $n = 5$, $p < 0.05$).

Discussion

There is an unmet need for the safer and more efficacious pharmacological management of AF with compounds that lack ventricular cardiac electrophysiological (proarrhythmic) adverse effects. In the majority of in vivo studies characterizing drug candidates against AF, animals anesthetized with volatile and (or) intravenous anesthetics were used. These anesthetic agents have their own relatively well identified effects on cardiac ion channels (Carnes et al. 1997; Heath and Terrar 1996; Morey et al. 1997; Pancrazio et al. 1993; Sakai et al. 1996) that can significantly influence the results of these antiarrhythmic studies (Freeman et al. 1990; Napolitano et al. 1996). In this work, the effects of the atrial selective $I_{K,ACh}$ inhibitor TQ on AF were investigated in freely moving, conscious dogs, and the results were compared with those with dofetilide and propafenone, drugs used in the clinical setting for rhythm control in patients with AF. Also, for the first time, the effects of these compounds on atrial action potential configuration and parameters were compared in right atrial trabeculae isolated from dogs with chronic right atrial tachypacing-induced AF.

Rapid atrial pacing in dogs is an established large animal AF model where tachypacing leads to electrical and structural remodeling in the atria (Morillo et al. 1995; Gaspo et al. 1997). In the present study, the electrical remodeling was monitored as the gradual decrease in AERP over the course of chronic tachypacing in our animals. In our conscious in vivo canine AF model, TQ markedly

and dose-dependently reduced the incidence of AF, the total and average duration of AF episodes, and this effect was paralleled by a significantly increased right AERP following acute intravenous TQ administration (Fig. 2). The significant prolongation by TQ of the APD at all percentages of repolarization was most likely responsible for the increased AERP in right atrial trabeculae isolated from these animals (Fig. 5). TQ is a honeybee venom toxin peptide derivative (Jin and Lu 1999) that is a highly selective inhibitor of GIRK (Kir3) channels carrying the acetylcholine-sensitive potassium current, $I_{K,ACh}$ (Dascal et al. 1993; Ehrlich et al. 2004). This channel is activated via muscarinic receptors following vagal stimulation (Yamada et al. 1998) leading to atrial action potential shortening and increased atrial dispersion of repolarization (Liu and Nattel 1997), suggesting an important role for this channel in creating an arrhythmia substrate for AF (Kovoor et al. 2001; Nattel 2002). Although $I_{K,ACh}$ downregulation was found in AF patients (Brundel et al. 2001; Dobrev et al. 2001), a constitutively active component independent of muscarinic receptor activation was later identified in patients with chronic AF (Dobrev et al. 2005). In a dog model of atrial tachypacing-induced AF, constitutive $I_{K,ACh}$ was also observed (Ehrlich et al. 2004). Inhibition of $I_{K,ACh}$ by TQ increased atrial APD in atrial tachycardia-remodeled canine coronary-perfused left atrial preparations and decreased atrial tachycardia inducibility (Cha et al. 2006), similarly to the APD prolongation observed in right atrial trabeculae and the in vivo antiarrhythmic activity following TQ application in our study. $I_{K,ACh}$ inhibition proved to be beneficial in previous, other canine models of AF — like aconitine and vagal nerve stimulation-induced AF (Hashimoto et al. 2006); however, in these studies, the effects of $I_{K,ACh}$ inhibition were tested during isoflurane and (or) combined isoflurane + thiopental anesthesia (Yamamoto et al. 2014). Thiopental significantly prolonged AERP in a concentration-dependent manner and caused an increase in atrial wavelength in guinea pig hearts (Napolitano et al. 1996), and isoflurane was found to have antifibrillatory effects in canine atria (Freeman et al. 1990). Although $I_{K,ACh}$ is also present in the ventricles (Krapivinsky et al. 1995), it is important to note that in conscious dogs, TQ did not prolong the QT interval in this study, suggesting that selective $I_{K,ACh}$ block is unlikely to provoke ventricular arrhythmias based on repolarization prolongation. The lack of QT prolongation by TQ in this study is in agreement with previous studies showing no significant ventricular effects following $I_{K,ACh}$ block (Machida et al. 2011).

The class IC antiarrhythmic drug propafenone and class III antiarrhythmic compound dofetilide were chosen as reference molecules in this study; both compounds are used in the clinical management of AF for rhythm control (Kirchhof et al. 2016; Piccini and Fauchier 2016). Both propafenone and dofetilide reduced AF incidence, decreased the duration of AF episodes, and increased right atrial ERP in conscious dogs with right atrial tachypacing-induced remodeling. Dofetilide prolonged the atrial APD while propafenone increased atrial conduction time in right atrial trabeculae isolated from dogs with AF. Dofetilide selectively blocks I_{Kr} in the concentration used in this study (Jurkiewicz and Sanguinetti 1993), and its beneficial effects in AF are based on prolongation of atrial repolarization and AERP (Allessie et al. 2001; Pedersen et al. 2001; Singh et al. 2000). However, dofetilide significantly prolongs ventricular APD as well that manifests as marked QT prolongation on the ECG, and can provoke serious ventricular arrhythmias (Wolbrette 2003; Lengyel et al. 2007). In the present study, dofetilide significantly prolonged the QT interval in conscious animals. Propafenone is a class IC antiarrhythmic drug, exhibiting I_{Na}, beta-adrenergic receptor and also HERG-blocking properties (Kohlhardt and Seifert 1980; Stoschitzky et al. 2016; Mergenthaler et al. 2001), and the drug is successfully applied for rhythm control in AF management due to its conduction-slowing effects (Allessie et al. 2001; Kirchhof et al. 2016). Propafenone is not recommended in patients with structural heart disease due to ventricular proarrhythmia and increased mortality (CAST Investigators 1989). Interestingly,

Fig. 4. Effect of propafenone (0.3 and 1 mg/kg, i.v.) administration on right atrial tachypacing-induced experimental atrial fibrillation (AF) in conscious dogs. The data show that propafenone administration significantly (A) reduced the incidence of AF, (B) increased the atrial effective refractory period (AERP), and decreased (C) the total duration of AF and (D) the average duration of AF episodes (only the larger dose) in conscious dogs. *$p < 0.05$; significantly different from control values, one-way ANOVA, $n = 6$ animals. AERP shown in the figure was measured at the basic cycle length of 300 ms. [Colour online.]
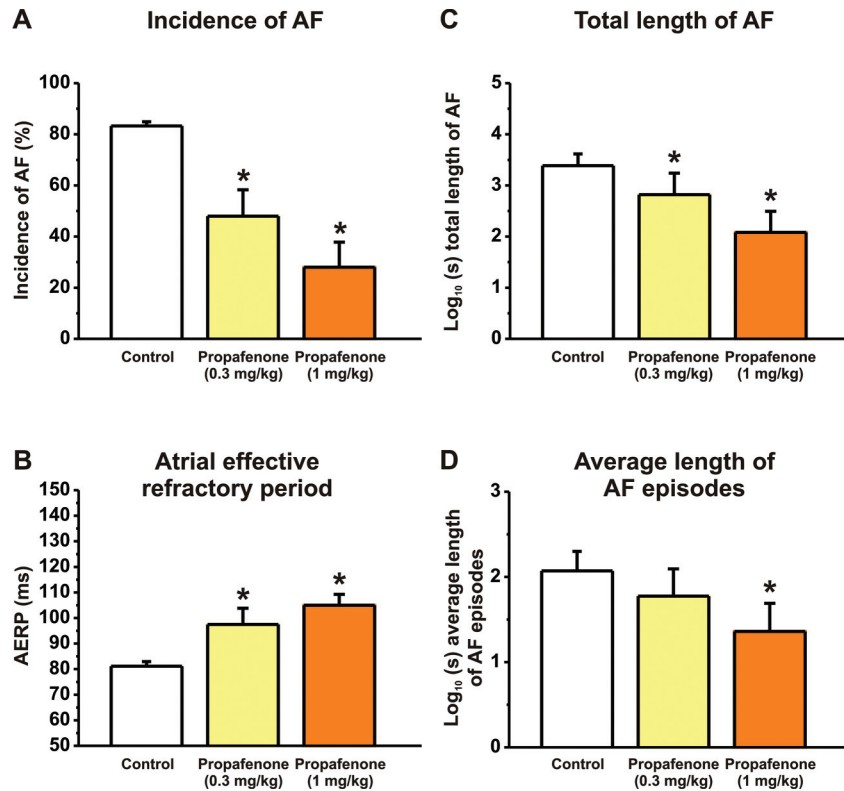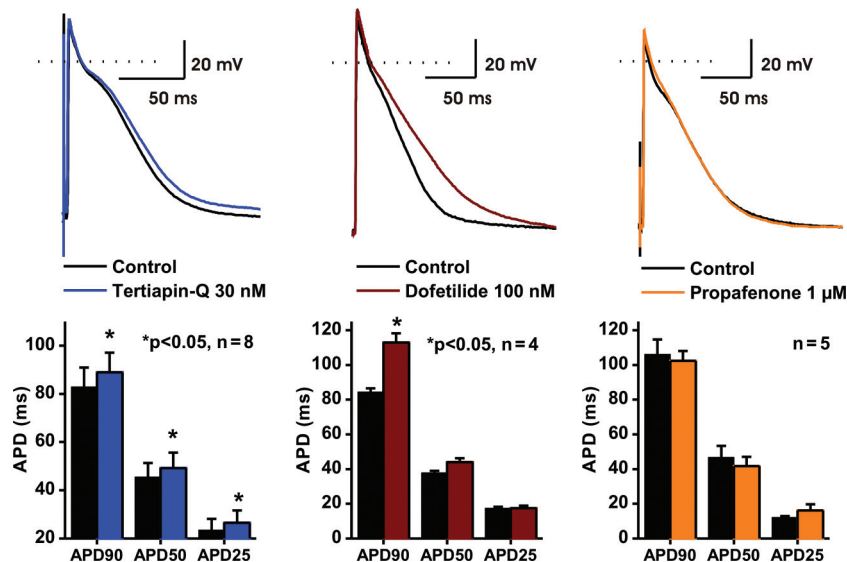

Fig. 5. Effects of tertiapin-Q (TQ; 30 nmol/L), dofetilide (100 nmol/L), and propafenone (1 µmol/L) on action potential durations (APD) at 90%, 50%, and 25% of repolarization, measured in right atrial trabeculae isolated from dogs with AF. Top panel shows representative AP recordings and bottom panel summarizes grouped data ($n = 4$–8/group). TQ prolonged APD measured at all % of repolarization. As expected, dofetilide only prolonged APD_{90} and propafenone did not influence APD. The dotted line in the top panel represents 0 mV.

the applied dose and concentration of propafenone did not prolong the QT interval in conscious dogs and did not prolong APD in isolated right atrial trabeculae (Fig. 5). Therefore, propafenone most likely exerted its beneficial effects against AF via mechanisms other than HERG block in this study. The prolongation of repolariza-

tion and slowing of conduction would prevent or decrease atrial reentry following the administration of dofetilide and propafenone, respectively. Interestingly, both propafenone and dofetilide were suggested to exert $I_{K,ACh}$-blocking effects (Mori et al. 1995; Voigt et al. 2010); however, it is not clear yet to what

degree these effects contribute to their beneficial effects in patients with AF. Of note, neither dofetilide nor the class IC antiarrhythmic drug flecainide had significant effects against AF in dogs anesthetized with the combination of thiopental and isoflurane (Yamamoto et al. 2014), emphasizing again the need for experiments in conscious animals.

Study limitations

The species differences regarding the relative roles of different atrial ionic currents, including $I_{K,ACh}$, in dogs and humans are not yet fully explored. In dogs subjected to chronic atrial tachypacing, a constitutive $I_{K,ACh}$ has been observed (Ehrlich et al. 2004) and a constitutively active $I_{K,ACh}$ has also been identified in patients with chronic AF (Dobrev et al. 2005), suggesting a potentially important role of $I_{K,ACh}$ in AF. However, the complex etiology, and the heterogeneous mechanisms responsible for the initiation and maintenance of AF in clinical settings, as opposed to chronic atrial tachypacing in dogs should be considered. Based on the above, the results obtained in the chronic atrial tachypacing-induced canine experimental AF model should be extrapolated to human clinical settings with caution and further studies are needed to evaluate the role of $I_{K,ACh}$ block in patients with AF.

Conclusions

We found that the selective $I_{K,ACh}$ inhibitor TQ significantly decreased the incidence of AF, reduced the duration of AF episodes, and prolonged AERP in conscious dogs with chronic right atrial tachypacing induced atrial remodeling. In this model, similar effects on AF and AERP were observed following the administration of the class IC antiarrhythmic drug propafenone, and the class III compound dofetilide, both used in the clinical management of AF. In right atrial trabeculae isolated from these dogs with AF, atrial APDs were prolonged by TQ and dofetilide, but not by propafenone, which increased atrial conduction time. Importantly, TQ did not affect the QT interval, suggesting that the beneficial effects against AF are not accompanied by adverse effects on ventricular repolarization; therefore, selective $I_{K,ACh}$ inhibitors may be promising atrial selective compounds in the future management of AF.

Acknowledgements

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