The Journal of Physiology

The ultrarapid delayed rectifier potassium current has important functional role in the repolarization reserve of canine and human ventricular muscle

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Handling Editors: Natalia Trayanova & Crystal Ripplinger

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP290706#support-information-section).

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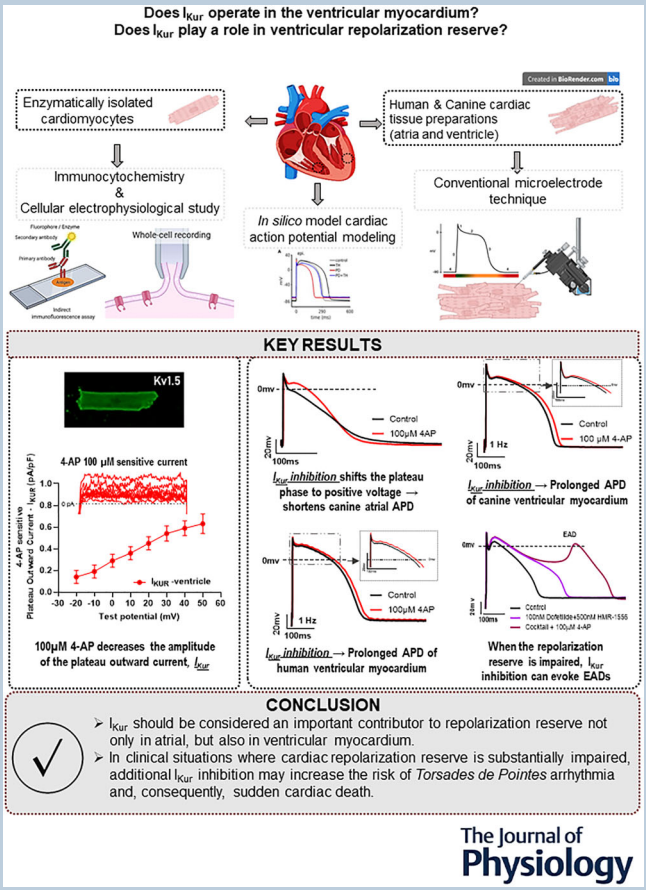
DOI: 10.1113/JP290706



Abstract figure legend The ultrarapid delayed rectifier potassium current (I_{Kur}) has long been considered an atrial-specific current with no functional role in the ventricles, despite evidence of its expression in ventricular myocytes. In the present study we challenged this prevailing concept and investigated the potential role of I_{Kur} in ventricular repolarization. Cellular electrophysiology and molecular cardiology experiments were performed on cardiac preparations obtained from dog and undiseased human hearts. We show clear evidences that I_{Kur} current is present in the ventricular myocardium at both molecular and functional levels. We confirm the presence of I_{Kur} current in atrial and ventricular tissue using immunochemistry, standard microelectrode and patch-clamp techniques. We also confirm that I_{Kur} current plays a significant role in ventricular repolarization reserve, as under conditions where the repolarization reserve was pharmacologically attenuated (by inhibiting I_{Kr} current), additive inhibition of I_{Kur} induces early afterdepolarizations (EADs), indicating that its blockade might have a significant proarrhythmic side effect.

Abstract The ultrarapid delayed rectifier potassium current (I_{Kur}) has long been considered an atrial-specific current with no functional role in the ventricles, despite evidence of its expression in ventricular myocytes. In the present study we challenged this prevailing concept and investigated the potential role of I_{Kur} in ventricular repolarization. Kv1.5 protein expression was determined by immunocytochemistry in canine and undiseased human cardiac tissue samples. Action potentials (APs) were recorded using conventional microelectrode techniques in canine and human cardiac preparations, and ion currents were measured using the whole-cell patch-clamp technique in isolated cardiomyocytes. *In silico* simulations were performed to investigate the role of the I_{Kur} current using a human ventricular model (T-World$_{IKur}$). Kv1.5 protein expression was detected in canine atrial, ventricular and Purkinje fibre cells, as well as in undiseased human left ventricular myocardium. In canine atrial preparations I_{Kur} inhibition shifted the plateau phase of the AP into the positive voltage direction and consequently shortened AP duration. Application of 100 µM 4-aminopyridine (4-AP) revealed ionic currents of similar magnitude in ventricular myocytes and Purkinje fibres, which were attributed to I_{Kur}. Inhibition of I_{Kur} caused prolongation of the APs

recorded from endocardial and midmyocardial preparations, as well as from Purkinje fibres. Under conditions of attenuated repolarization reserve I_{Kur} inhibition induced augmented repolarization lengthening and frequent early afterdepolarizations (EADs). *In silico* modelling data regarding the I_{Kur} inhibition-evoked EAD formations are in good agreement with the experimental findings. I_{Kur} is present and functionally active in ventricular myocardium and may represent a significant contributor to ventricular repolarization reserve.

(Received 16 February 2026; accepted after revision 11 June 2026; first published online 29 June 2026)
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Key points

- The ultrarapid delayed rectifier potassium current (I_{Kur}) has long been considered an atrial-specific current with no functional role in the ventricles.
- In the present study, we challenged this prevailing concept and investigated the potential role of I_{Kur} in ventricular repolarization. Cellular electrophysiology and molecular cardiological experiments were performed on cardiac preparations obtained from dog and undiseased human hearts.
- We present clear evidence that I_{Kur} current is present in the ventricular myocardium at both molecular and functional levels. We also confirm that I_{Kur} current plays a significant role in ventricular repolarization reserve.
- We concluded that I_{Kur} is present and functionally active in ventricular myocardium and may represent a significant contributor to ventricular repolarization reserve.

Introduction

The cardiac action potential (AP) is regulated by multiple, simultaneously operating transmembrane ion channels (Varro et al., 2021). During the repolarization phase potassium channels work in parallel and interact with each other. Importantly in certain pathophysiological settings they can compensate for the loss of function of other channels that conduct repolarizing current, providing safer repolarization, a concept called 'repolarization reserve' (Roden, 1998, 2006; Varro et al., 2021). The expression patterns of these ion channels show large species- and tissue-specific differences resulting in a large variability in the shape of the AP (Varro et al., 2021). Cardiac repolarization and, consequently, the effective refractory period (ERP) are partially controlled by different outward potassium currents; therefore their inhibition, which results in repolarization and ERP prolongation, is considered an important antiarrhythmic mechanism (Varro & Baczko, 2011), often called class III antiarrhythmic action defined by the classification of Vaughan Williams (Lei et al., 2018; Singh & Vaughan Williams, 1970).

However, antiarrhythmic drugs with this mode of action, in addition to exerting antiarrhythmic effects, due to prolonged ventricular repolarization and increased dispersion of repolarization, can elicit proarrhythmic side effects (Wang et al., 2023), greatly limiting their clinical use. In an early study of the guinea pig ventricle a very rapidly activating and non-deactivating potassium current was described in the plateau voltage range, which was called the iKp 'plateau potassium current' (Yue & Marban, 1988). Later in the 1990s several studies revealed the existence of a potassium current, known as the ultrarapid delayed rectifier potassium current (I_{Kur}), in canine (Fedida et al., 1993; Yue et al., 1996) and human atrial muscle (Amos et al., 1996; Feng et al., 1996), conducted by Kv1.5 channels. These early studies – although showing Kv1.5 protein expression in both canine (Eldstrom et al., 2006) and human ventricle (Amos et al., 1996; Feng et al., 1996; Mays et al., 1995) – did not reveal a functional role for I_{Kur} (Amos et al., 1996). Therefore it was assumed that I_{Kur} inhibition could result in an atrial-selective antiarrhythmic effect free of the proarrhythmic complications in the ventricle (Ravens & Wettwer, 2011). This concept paved the way for the development of a large number of purportedly atrial-selective drugs (Ford et al., 2016; Gogelein et al., 2004; Regan et al., 2006), some of which entered the clinical phase of development (Camm et al., 2019; Ford et al., 2013; Shunmugam et al.,

2018). However, in 2007 and 2008 two elegant studies (Sridhar et al., 2007; Sridhar et al., 2008) from the same research group described the functional existence of I_{Kur} in canine ventricular muscle. This promptly evoked a commentary article by Wettwer, published in the *British Journal of Pharmacology*, concluding that 'the presence or absence of I_{Kur} in ventricular tissue needs further experimental investigation, especially in human myocardium' (Wettwer, 2007). In spite of the published results and this commentary no further studies were initiated in this regard. However, during subsequent years the scientific community and the literature did not attribute notable significance to this concern; thus the usefulness of the atrial-selective concept of I_{Kur} inhibition for drug development was widely accepted (Calvo et al., 2018; Ravens & Wettwer, 2011; Ravens et al., 2013; Schmitt et al., 2014; Voigt & Dobrev, 2016). In addition several I_{Kur} inhibitor drug developmental projects (Ford et al., 2013; Gogelein et al., 2004) were carried out or continued, including clinical investigations (Camm et al., 2019; Shunmugam et al., 2018).

Because the fine-tuning of the cardiac ventricular repolarization and the complexity of repolarization reserve are still not fully understood, our present study, following the suggestion made in the commentary of Wettwer (Wettwer, 2007), investigated the expression and possible function of I_{Kur} in canine and human ventricular muscle preparations.

Methods

Ethical approvals

We confirm that our study, conducted using both animal and human tissue samples, complies with the ethical principles and policies of *The Journal of Physiology* and is compliant with reporting requirements detailed in the journal's latest Editorial on ethics and welfare (O'Halloran, 2024).

Animal ethics statements

The experiments, conducted in ventricular preparations isolated from canine hearts, were carried out in compliance with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication number 85-23, revised 1996), with the ARRIVE guidelines (Percie du Sert et al., 2020), and conformed 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-74-24-2017), along with the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval number: XIII/3331/2017).

Patient ethics statements

Undiseased human myocardial samples were obtained from general organ donors with no previous history of cardiovascular disease whose hearts could not have been used for transplantation due to logistical reasons. The investigations were conducted in accordance with the ethical principles laid down in the Helsinki Declaration, and the sample collection and all experimental protocols were approved by The Scientific Board at the Hungarian Ministry of Health (ETT-TUKEB: 4991–0/2010-1018EKU). Organ donor samples were procured under Hungarian national presumed-consent legislation in accordance with ETT-TUKEB regulations. Proper consent was obtained for the use of each individual's tissue for experimentation.

Animal experiments

Beagle dogs of both sexes, weighing 11–13 kg, were used in the experiments. The animals were purchased from a local experimental animal breeder certified by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development, Hungary (András Tóth, Ásotthalom, Hungary; breeder's authority approval number: XXXV/2018). Animal housing and handling were in accordance with good animal practice as defined by the Federation of European Laboratory Animal Science Association (FELASA), the ARRIVE guidelines (Percie du Sert et al., 2020) and the recommendations of *The Journal of Physiology* (O'Halloran, 2024). Dogs were housed in kennels that fulfilled legal requirements regarding size and environmental enrichment, including elevated resting places for each dog. The animals were kept at standard room temperature, humidity and lighting. Food was provided twice a day, and drinking water was given *ad libitum*.

The animals and the isolation of the ventricular myocytes and Purkinje cells

Ventricular cardiomyocytes and Purkinje cells were enzymatically dissociated from the hearts of Beagle dogs. Hearts were removed following induction of anaesthesia. Anaesthesia was induced with 0.5 μg/kg intravenous sufentanyl (Sufentanil Torrex 5 μg/mL; Chiesi Pharmaceuticals GmbH, Vienna, Austria) as premedication, followed by 150 mg/kg intravenous pentobarbital (Release 300 mg/mL; WDT, Garbsen, Germany) induction. The depth of anaesthesia, as in most

human anaesthesia practices, was monitored based on the elicitation of autonomic and somatic reflexes induced by painful stimuli. The agent used for induction (in this case pentobarbital) was administered until the eyelash reflex disappeared. The quality and depth of analgesia were assessed by the absence of autonomic reflex responses to painful stimuli: in response to nociceptive input (e.g. surgical incision) there was no increase in blood pressure or heart rate, and both parameters remained stable and regular. The depth of anaesthesia was also indicated by the absence of the flexor reflex. Respiratory rate and pain-induced changes in respiration were not evaluated, as the animals were mechanically ventilated and did not exhibit spontaneous breathing. Parameters commonly used in human medicine to monitor the depth of anaesthesia, such as entropy and the bispectral index (BIS), were not assessed in this study due to technical limitations. In cases where any autonomic reflex response to painful stimuli was observed (e.g. an increase in heart rate or blood pressure), the dose of sufentanyl was increased.

After the induction of complete anaesthesia a left thoracotomy was performed in 11–13 kg Beagle dogs of both sexes. The animals were endotracheally intubated and mechanically ventilated (UGO Basile S.R.L. respirator; Biological Research Apparatus, VA, Italy). After left thoracotomy the heart was rapidly removed and placed into 4°C Locke solution. Overall 10 healthy animals from both sexes were used for the *in vitro* electrophysiological experiments. All parts of their hearts were utilized.

The method of enzymatic dissociation of ventricular midmyocardial myocytes from canine hearts was previously described (Topal et al., 2021).

Canine Purkinje fibre (PF) cells were isolated using a modified protocol described previously (Xiao et al., 2013). Free-running PF false tendons were excised from both ventricles and immediately placed in cold, calcium-free isolation solution supplemented with heparin. The tissue samples were then transferred to a 37°C oxygenated water-bath and washed for 5 min in Ca^{2+}-free isolation solution.

Enzymatic digestion was initiated with elastase (1.6 U/mL; Worthington) for 10 min, followed by collagenase (765 U/mL; Worthington, Type II) in isolation solution containing 33 μM Ca^{2+} for 20 ± 10 min. During digestion the fibres were gently agitated by magnetic stirring and continuously bubbled with oxygen.

After digestion PF cells were transferred back to Ca^{2+}-free isolation solution. The Ca^{2+} concentration was then gradually increased to a final concentration of 1 mM to allow the cells to readapt to physiological conditions.

Immunofluorescent detection of Kv1.5 in isolated canine and human cardiomyocytes

Kv1.5 protein expression was determined in enzymatically isolated myocytes from the right atrium, left ventricle and PFs of canines, as well as from the left ventricle of human donor hearts (Fedida et al., 2003; Nagy et al., 2009; Seprényi et al., 2006). Isolated cells were plated onto glass coverslips, fixed with acetone and then rehydrated in phosphate-buffered saline (PBS). Plasma membranes were stained with wheat germ agglutinin-Texas Red (1:500) for 30 min at room temperature. After PBS washes cells were blocked with 2.5% bovine serum albumin (BSA) in PBS+0.1% Tween-20 for 1 hour at room temperature. Cells were then incubated overnight at 4°C with either the primary antibody (anti-Kv1.5, Alomone Labs, Cat# APC-004; 1:50 in 1% BSA-PBS) or 1% BSA-PBS alone as a negative control (no primary control). After PBS washes cells were incubated with the secondary antibody (Alexa Fluor 488, Thermo Fisher Scientific, Cat# A-11034; 1:500 in 1% BSA-PBS) for 1 hour at room temperature. Finally cells were mounted on glass slides and imaged using a Zeiss LSM 880 laser scanning confocal microscope with a 40× water-immersion objective. Integrated densities were measured using a built-in plugin in ImageJ2 (FIJI) and plotted.

Voltage-clamp measurements

One drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope (Olympus IX51, Olympus, Tokyo, Japan), and individual myocytes were allowed to settle and adhere to the chamber bottom for at least 5–10 min before superfusion was initiated and maintained by gravity. Only rod-shaped cells with clear striations were used. HEPES-buffered Tyrode's solution (composition in mM: NaCl 144, NaH_2PO_4 0.4, KCl 4.0, $CaCl_2$ 1.8, $MgSO_4$ 0.53, glucose 5.5 and HEPES 5.0, at pH of 7.4) served as the normal superfusate.

The external solution contained 1 μM nisoldipine (Bayer AG, Germany) to completely block L-type $Ca^{2\pm}$ current (I_{CaL}). When measuring I_{Kr} and I_{Ks} currents, the current components were separated pharmacologically by adding 500 nmol/L HMR-1556 (Biosynth Ltd, UK, selective I_{Ks} blocker) or 100 nmol/L dofetilide (Biosynth Ltd, UK, selective I_{Kr} blocker), respectively, to the external solution. In these measurements the inward $Na^{\pm}$ current (I_{Na}) was inactivated using a short prepulse to −40 mV, which also largely inactivated the transient outward current (I_{to}).

Micropipettes were fabricated from borosilicate glass capillaries (Science Products GmbH, Hofheim,

Germany) using a P-97 Flaming/Brown micropipette puller (Sutter Co, Novato, CA, USA) and had a resistance of 1.5–2.5 MΩ when filled with pipette solution. The membrane currents were recorded using Axopatch-200B amplifiers (Molecular Devices, Sunnyvale, CA, USA) in the whole-cell configuration of the patch-clamp technique. The membrane currents were digitized at 250 kHz using analogue-to-digital converters (Digidata 1440A, Molecular Devices, USA) under software control (pClamp 10, Molecular Devices, USA). All experiments were carried out at 37°C.

Cardiac tissue preparations

Papillary muscle or trabecular preparations were obtained from the right ventricle of canine hearts. Midmyocardial tissue slices were prepared using a vibratome. Briefly a tissue block of approximately 1 cm^3 was excised from the left ventricle, and the epicardial surface was glued onto the specimen platform of the vibratome chamber (Vibratome 300 Sectioning System, Ted Pella Inc., CA, USA) using Histoacryl tissue adhesive (Braun, Melsungen, Germany). The chamber was filled with modified Locke's solution (in mM: 130 NaCl, 21.5 $NaHCO_3$, 4.5 KCl, 12 glucose, 0.4 $MgCl_2$, 1.8 $CaCl_2$, 10 2,3-butanedione monoxime; pH 7.35 ± 0.05).

Slices of 400 μm thickness were cut at a forward speed of 0.05 mm/s with a lateral blade vibration amplitude of 2 mm. The slices were allowed to recover for 2 hours in the same solution, after which they were transferred to standard Locke's solution (without 2,3-butanedione monoxime) for additional 2 hours under continuous carbogen (95% O_2, 5% CO_2) bubbling.

AP measurements in multicellular preparations of canine and human cardiac tissue

APs of all types of preparations were obtained from cardiac muscle using the conventional microelectrode technique. APs were recorded from left atrial, right ventricular trabecular or papillary muscle (Orvos et al., 2020) and left ventricular slice preparations obtained from canine hearts. Briefly the preparations were individually mounted in a tissue chamber with a volume of 50 ml of modified Locke's solution which contained (in mM) NaCl 128.3, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.42, $NaHCO_3$ 21.4 and glucose 10. When gassed with a mixture of 95% O_2 and 5% CO_2 at 37°C, the pH of this solution was adjusted between 7.35 and 7.4.

APs of human myocardium were recorded in right ventricular trabeculae and papillary muscle preparations (<2 mm in diameter; $n = 45$) of 10 undiseased human donor hearts. After explantation each heart was perfused with cardioplegic solution and kept cold (4–6°C) for 2–4 h before dissection. Trabeculae and papillary muscles were then excised and mounted in a tissue chamber (volume, ≈50 mL) perfused with oxygenated (95% O_2+5% CO_2) modified Tyrode's solution containing (in mmol/L) NaCl 115, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 20 and glucose 11. The pH of this solution was maintained at 7.35–7.45 at 37°C.

Each preparation was stimulated through a pair of platinum electrodes in contact with the tissue using rectangular current pulses of 1–3 ms duration at twice the threshold strength at a constant basic cycle length of 1000 ms for ventricular preparations. These stimuli were delivered 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 M KCl and having tip resistances of 5–20 MΩ, connected to the input of a high-impedance electrometer (Experimetria, type 309, Budapest, Hungary), which was coupled to a dual-beam oscilloscope.

The AP duration (APD), measured at 25%, 50%, 70% and 90% of repolarization, was determined using an in-house developed software (APES) running on a computer equipped with an ADA 3300 analogue-to-digital data acquisition board (Real Time Devices, Inc., State College, PA, USA) having a maximum sampling frequency of 40 kHz.

Attempts were made to maintain the same impalement throughout each experiment. If an impalement became dislodged, adjustment was attempted, and if the AP characteristics of the re-established impalement deviated by less than 5% from the previous measurement, the experiment continued.

Data analysis

IBM SPSS Statistics V25, Microsoft Excel (Microsoft Office Professional Plus 2016) and Origin software packages were used for statistical analysis. All data are expressed as means ± SD. The '*n*' number refers to the number of experiments (i.e. the number of cells in case of patch-clamp and the number of ventricular muscle preparations – papillary or trabecular muscle – in case of AP measurements), whereas the '*N*' number refers to the number of animals or organ donor hearts, respectively.

Before statistical testing data distribution was assessed for normality using the Shapiro–Wilk test, and homogeneity of variances was evaluated using variance equality tests. Comparisons between two groups under a single condition were performed using either a paired or an unpaired two-tailed Student's *t* test, depending on the experimental design. A paired *t* test was applied when measurements were obtained from the same preparation under control and 4-aminopyridine (4-AP) treated conditions, whereas an unpaired *t* test was

used for comparisons between independent groups. For experiments involving repeated measurements obtained from the same preparation across different conditions two-way repeated-measures ANOVA was applied. When a significant overall effect was detected, *post hoc* multiple comparison tests with appropriate correction were used to determine differences at individual data points. Data were considered statistically significant when $^{*}P \leq 0.05$.

Computer simulations

The midmyocardial version of the T-World computer model of a human ventricular myocyte (Tomek, Holmes, Bury et al., 2026; Tomek, Holmes, Martinez-Navarro et al., 2026) was used throughout the study. To investigate the role of I_{Kur}, we incorporated the formulation of the current from the Grandi et al. (2011) atrial myocyte model (Grandi et al., 2011). Conductance Gkur was set to 0.0035 mS/μF, and the addition of a new potassium current was offset by reducing T-World's GKb conductance by 43% to 0.0062 mS/μF, resulting the model termed 'T-World$_{IKur}$'.

To investigate the role of I_{Kur} in EAD promotion, we simulated a population of 786 cells used in T-World (Tomek, Holmes, Bury et al., 2026; Tomek, Holmes, Martinez-Navarro et al., 2026), which varies key ionic currents between 67% and 150% and calibrates the endocardial variant to human biomarkers as described in the original publication. In each of those 786 cells we compared EAD occurrence under the following conditions:

(1) T-World with I_{Kur}.
(2) The same model with additional I_{Kr} and I_{Ks} inhibition (both blocked by 66%, i.e. reduced to 34%).
(3) The model with I_{Kr} and I_{Ks} partly inhibited as above, with additional full block of I_{Kur}.

Results

Kv1.5 protein expression in various canine myocytes and human left ventricular myocytes

The Kv1.5 channel protein, considered the alpha subunit of the channel, expressed in mammalian cell lines carries a current strikingly similar to the I_{Kur} measured in native atrial myocytes (Yue et al., 1996). The experimental design aimed to investigate the possible expression of Kv1.5 protein in different myocytes of canine origin and, additionally, in human ventricular myocytes. As Fig. 1 indicates the immunocytochemistry studies

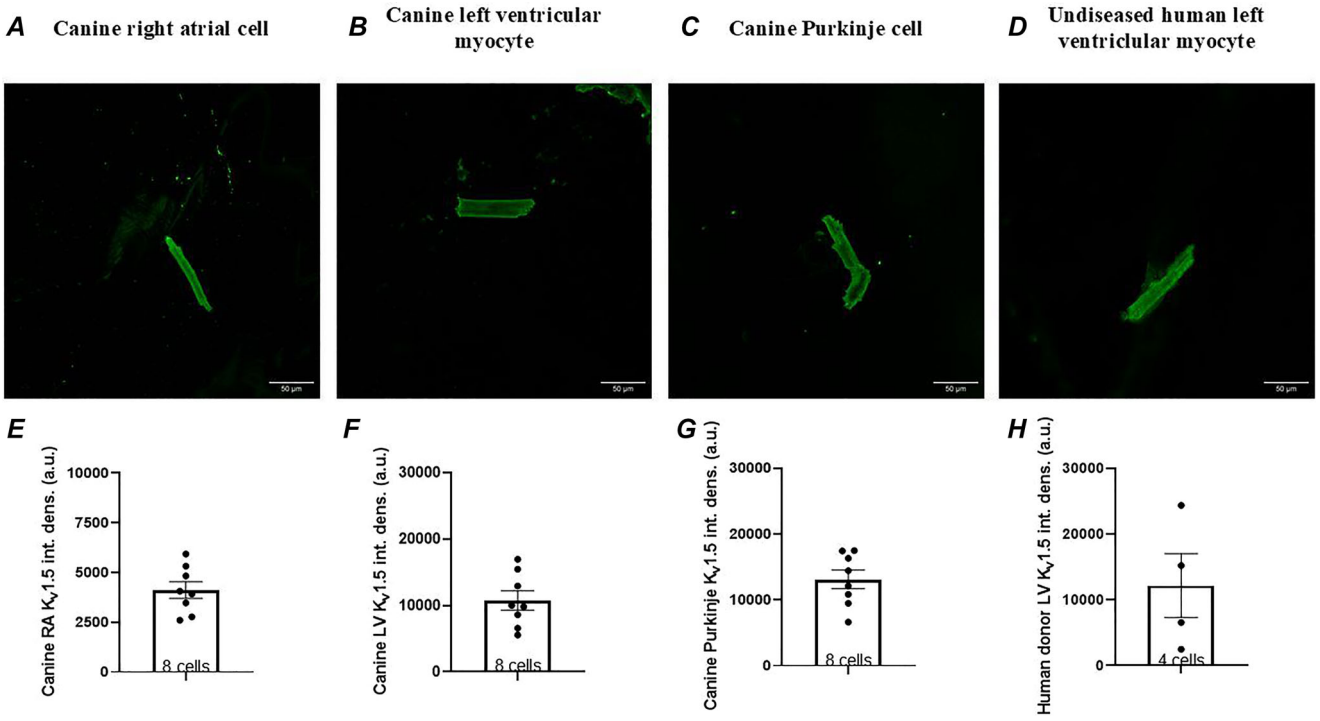

Figure 1. Kv1.5 protein expression determined by immunocytochemistry in enzymatically isolated cardiomyocytes
The representative original immunofluorescence images show expression of Kv1.5 protein in isolated (*A*) canine right atrial, (*B*) canine left ventricular myocytes, (*C*) canine Purkinje cells and (*D*) left ventricular myocytes obtained from undiseased human hearts. *E–H*, relative density of Kv1.5 protein immunolabeling obtained from enzymatically isolated cardiac cells. Data are expressed as means ± SD.

confirmed the marked presence of Kv1.5 channel protein in both atrial, ventricular and Purkinje native myocytes, respectively.

Effect of I_{Kur} inhibition on canine cardiac atrial APs

In spite of the consistent published results that I_{Kur} in the atria is abundantly expressed and results in large I_{Kur} the data regarding the effect of I_{Kur} inhibition on atrial repolarization are still controversial. Some studies reported a significant increase in atrial APD after I_{Kur} inhibition (Ford et al., 2016; Xing et al., 2009), whereas others reported no change or a substantial change in canine or human atrial muscle (Burashnikov & Antzelevitch, 2008; Loose et al., 2014). Therefore, in our present study we investigated the effect of full I_{Kur} inhibition by 100 μM 4-AP in dog left atrial preparations.

As Fig. 2*A* and *B* shows in a representative original AP recording and in the summary bar graph, 100 μM 4-AP moderately but significantly shortened atrial APD_{90}. However, the plateau phase of the AP was significantly lengthened, reflected as an increase in APD_{25}. Also the voltage of the plateau phase was shifted markedly into the positive direction. However, in those experiments where I_{Kr} was previously inhibited by 100 nM dofetilide, additional application of 100 μM 4-AP significantly prolonged APD_{25}, APD_{75} and APD_{90} (Fig. 2*C* and *D*).

Identification of I_{Kur} in canine Purkinje and ventricular myocytes

I_{Kur} was measured in left ventricular and Purkinje myocytes by the patch-clamp technique. To determine the current, a certain I_{Kur} inhibitor (100 μM 4-AP) and unique voltage protocols were applied (Fig. 3). As the insets of Fig. 3*A*–*C* show the cells were held at −80 mV and a 100 ms prepulse to +30 mV was applied to inactivate the transient outward potassium current. Then voltage was returned to −40 mV for 10 ms, followed by 300 ms test pulses from −20 to +50 mV with a pulsing cycle length of 3 s. After 10 min of equilibrium 100 μM 4-AP was applied for 5 min.

The representative recordings of ventricular myocytes clearly demonstrate a prominent plateau outward current,

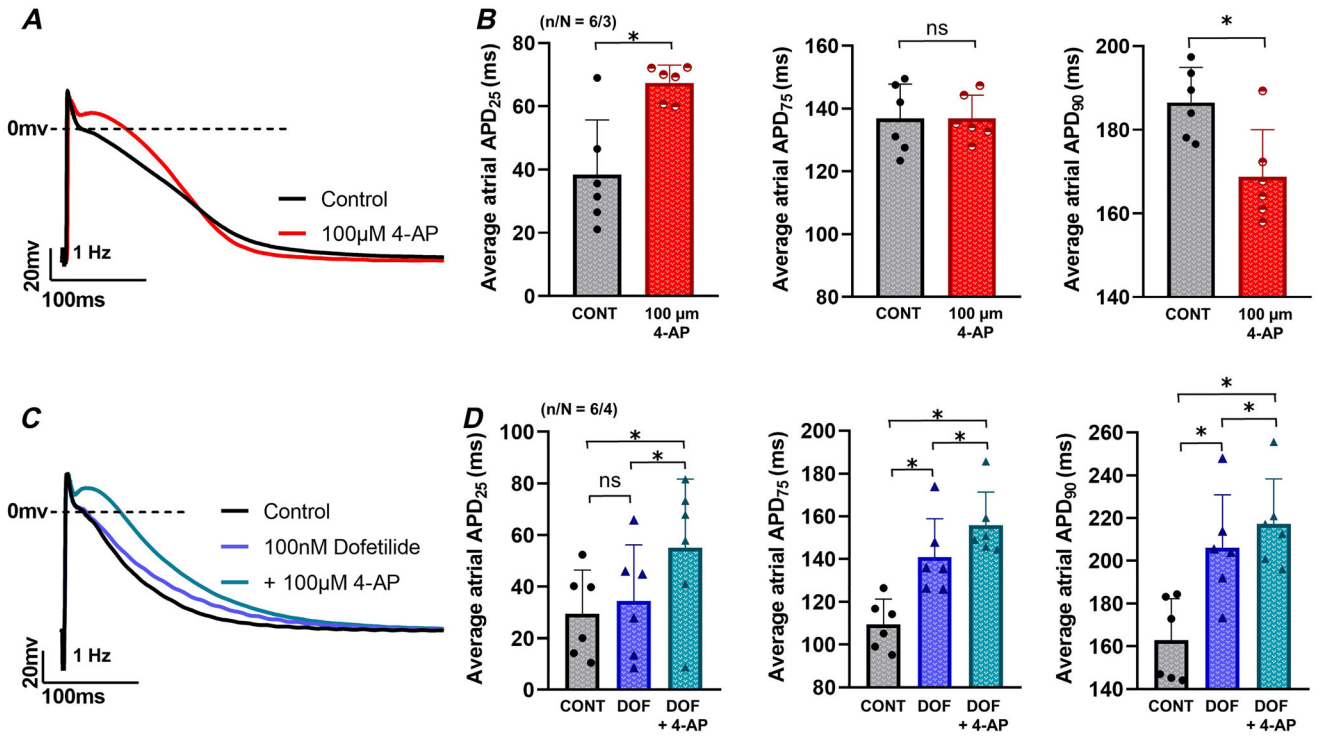

Figure 2. Effect of acute exposure to 100 μM 4-AP on the action potential (AP) parameters in atrial tissue preparations
A, representative original atrial action potential recording shows the 100 μM 4-AP-induced action potential changes in control conditions and after application of the drug. The bar graphs with individual data points show average APD_{25}, APD_{75}, APD_{90} parameters, respectively. *B* and *C*, the representative original action potential recording introduces the prolongation effect of 100 μM 4-AP after attenuated repolarization reserve with 100 nM dofetilide. *D*, the combination of 100 nM dofetilide and 100 μM 4-AP leads to significant prolongation of all the investigated AP duration (APD) parameters. Data are expressed as means ± SD. *n/N* represents the number of myocytes (*n*) and the number of experimental animals (*N*).

the amplitude of which is significantly decreased after the application of 100 μM 4-AP. The 4-AP-sensitive outward potassium current was obtained by digital subtraction (Fig. 3*A*). As Fig. 3*A* (middle panel) and Fig. 3*B* indicate 100 μM 4-AP significantly decreased the amplitude of the outward potassium current in isolated ventricular midmyocardial cells, which starts to activate at −20 mV and then perceptibly increases with higher positive test potentials up to + 50 mV (Fig. 3*C*). At +20 the amplitude of the current was 68.93 ± 10.18 pA. The capacitance of the myocytes was 155.20 ± 9.14 pF (n/N = 12/7). In further experiments AP-like voltage pulses were applied with a pulsing cycle length of 1 s. After control measurements 4-AP was applied. As Fig. 3*D* shows the 4-AP-sensitive difference current indicated significant outward current during the plateau phase of the AP-like pulse.

Under the same circumstances the experiments were performed in isolated Purkinje cells. As the original recordings indicate in Fig. 4*A* the properties of the potassium current resemble those previously described for ventricular myocytes. The current density–voltage relationship of the 100 μM 4-AP-sensitive current of left ventricular midmyocardial myocytes is shown in Fig. 4*B*. The I/V curve of the 4-AP-sensitive potassium shows that the current – similar to the results observed in the ventricle – starts to activate at −20 mV (Fig. 4*C*).

In addition the selectivity of 4-AP was tested at the applied concentration in our own experiments on other outward potassium currents, which play important roles in cardiac ventricular repolarization. 100 μM 4-AP had no or negligible effect on the other outward potassium currents such as the rapid (I_{Kr}) and the slow (I_{Ks}) delayed rectifier and inward rectifier (I_{K1}) potassium currents and elicited a minimal 14.2% decrease in the transient outward (I_{to}) potassium current without changing its inactivation kinetics (Fig. 5).

Effect of I_{Kur} inhibition on canine cardiac ventricular and PF APs

The influence of I_{Kur} inhibition on cardiac ventricular APs was studied in canine right ventricular papillary muscle, left ventricular midmyocardial tissue slices and PF preparations using the conventional microelectrode technique.

As illustrated in the panels of Fig. 6*B* and *E* 100 μM 4-AP increased the APD measured at 90% level of repolarization (APD_{90}) in right ventricular subendocardial preparations at a pacing cycle length of 1 Hz (235.1 ± 4.0 to

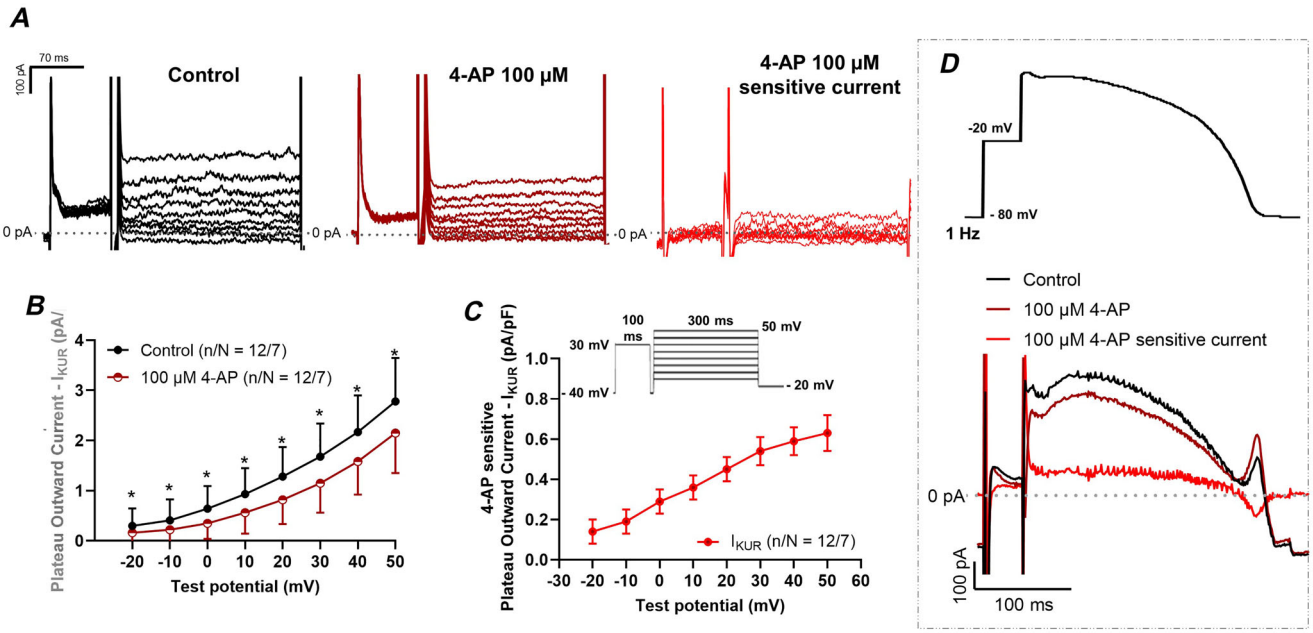

Figure 3. Effect of 100 μM 4-AP on the plateau outward current I_{Kur} in left ventricular midmyocardial myocytes
A, the original recordings show the control current amplitude in the current–voltage relationship (black recording), the inhibitory effect of 100 μM-AP (deep red recording) and the 4-AP-sensitive outward potassium current (bright red recording). *B* and *C* show the current–voltage relationship curves for I_{Kur} under control conditions and after application of 100 μM 4-AP, as well as the 4-AP-sensitive current. Insets indicate the voltage protocols. The holding potential (HP) of the cells is −40 mV. *D*, the response for action potential-like voltage pulses with a pulse cycle length of 1 s in control conditions, after superfusion with 100 μM 4-AP and 4-AP-sensitive current. Data are expressed as means ± SD. *n/N* represents the number of myocytes (*n*) and the number of experimental animals (*N*).

246.9 ± 5.6 ms, CL = 1000 ms, $n = 5$, $^{*}P = 0.001$, Fig. 6*B*) and in left ventricular midmyocardial slice preparations (273.2 ± 12.8 to 288.3 ± 14.4 ms $n = 7$, CL = 1000 ms, $^{*}P = 0.002$, Fig. 6*E*) as well. In addition to evoking lengthening of repolarization 4-AP led to a shift in the plateau voltage towards more positive potentials in all the examined ventricular preparations (Fig. 6*A* and *D*). In all ventricular preparations the APD_{90} and the effect of 4-AP showed significant cycle length dependency at all pacing frequencies (Fig. 6*C* and *F*).

In PFs 100 μM 4-AP significantly prolonged the APD_{90} (360.8 ± 17.7 to 403.4 ± 25.2 ms, CL = 1000 ms, $n = 5$, $^{*}P = 0.001$, Fig. 6*H*). The cycle length-dependent protocol also revealed that 100 μM 4-AP prolonged APD_{90} at all pacing frequencies (i.e. the effect was also cycle length dependent, Fig. 6*I*).

Effect of I_{Kur} inhibition on human cardiac ventricular APs

The effects of 50 and 100 μM 4-AP at a standard stimulation frequency of 1 Hz were investigated in right ventricular papillary and trabecular cardiac muscle preparations of human donors (Fig. 7). As the representative original recordings indicate both concentrations of 4-AP in healthy human ventricular preparations led to the prolongation of the AP and a shift in the plateau voltage to a positive direction (Fig. 7*A* and *C*). The inhibition of I_{Kur} induced prolongation of APD_{90} as observed in the canine ventricular muscle at the pacing cycle length of 1000 ms (Fig. 7*B* and *D*). The applied two different concentrations induced a similar APD_{90} prolongation that did not differ significantly from each other.

Effect of I_{Kur} inhibition on canine cardiac ventricular APs with impaired repolarization reserve

In a series of experiments in left ventricular slice preparations, APD_{90} was substantially lengthened due to a significant reduction in repolarization reserve by simultaneous inhibition I_{Kr} and I_{Ks} with 100 nM dofetilide and 500 nM HMR-1556, respectively (Fig. 8*A* and *B*). In these experiments 100 μM 4-AP prolonged APD_{90} significantly more than in preparations with normal repolarization reserve (Fig. 8*D*). In two out of five experiments with 100 μM 4-AP in addition to

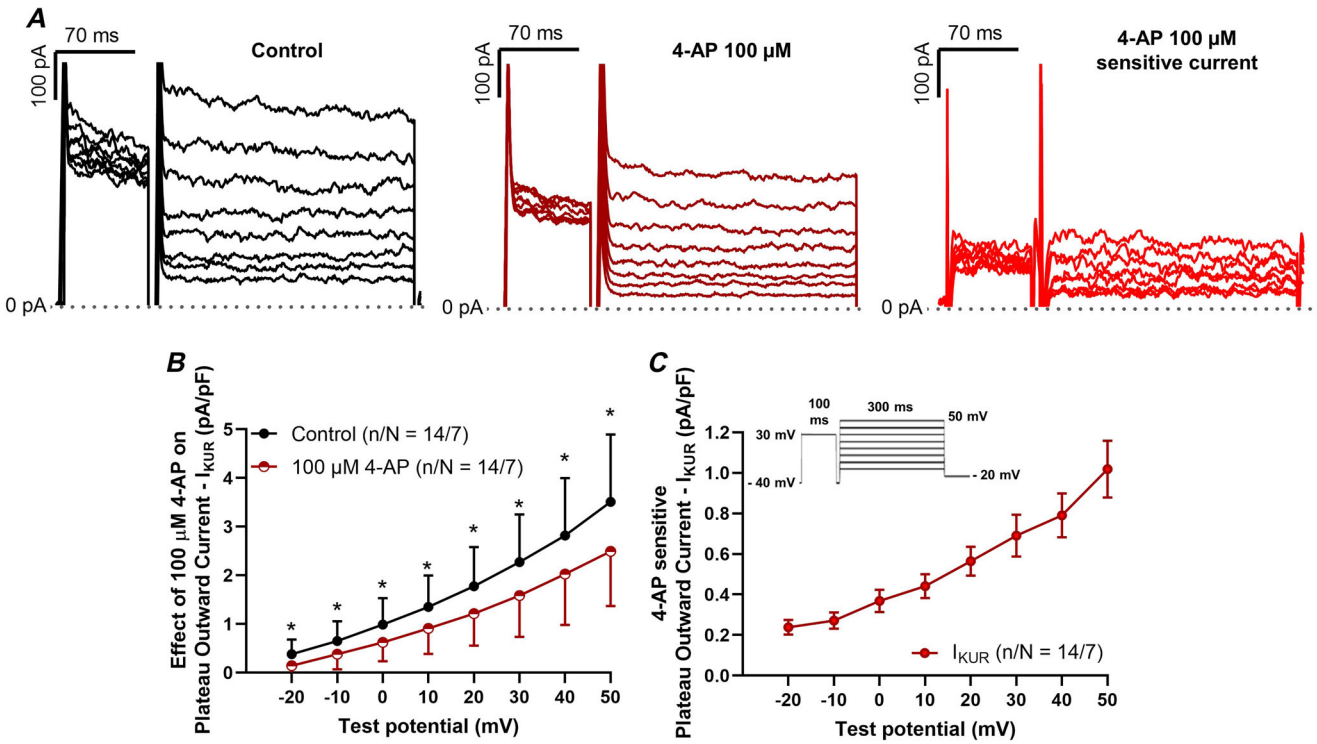

Figure 4. Effect of 100 μM 4-AP on the plateau outward current I_{Kur} in Purkinje cells
A, the original recordings show the control current amplitude in the current–voltage relationship (black recording), the inhibitory effect of 100 μM-AP (deep red recording) and the 4-AP-sensitive outward potassium current (bright red recording). *B* and *C* show the current–voltage curves for I_{Kur} under control conditions and after application of 100 μM 4-AP, as well as the 4-AP sensitive current. Insets indicate the voltage protocols. The holding potential (HP) of the cells is −40 mV. Data are expressed as means ± SD. *n/N* represents the number of myocytes (*n*) and the number of experimental animals (*N*).

the repolarization prolongation early afterdepolarizations (EADs) were observed (Fig. 8*C*).

In silico investigation of the effect of I_{Kur} inhibition on human cardiac ventricular AP model with impaired repolarization reserve

Finally we investigated the potential role of I_{Kur} block on ventricular repolarization and in EAD formation using T-World$_{IKur}$, a modification of the T-World human ventricular myocyte model (Tomek, Holmes, Bury et al., 2026; Tomek, Holmes, Martinez-Navarro et al., 2026) with I_{Kur} incorporated, based on the Grandi-Bers model (Grandi et al., 2011). Consistent with our experimental observations I_{Kur} inhibition modestly extends the APD (Fig. 9*A*).

To test whether this AP prolongation may translate into an increased EAD risk we simulated a population of models varying the conductance of key ionic currents (see Methods), comparing control midmyocardial cells, cells with induced long QT (66% inhibition of I_{Kr} and I_{Ks}) and long QT with additional full inhibition of I_{Kur}. Although no controls showed EADs at 1 Hz stimulation, EADs were present in 80 cells with $I_{Kr}+I_{Ks}$ inhibition and 96 cells with $I_{Kr}+I_{Ks}+I_{Kur}$ inhibition. This confirms that I_{Kur} inhibition has the potential to promote EAD formation in the setting of reduced repolarization reserve (see example in Fig. 9*B*).

Discussion

The main message of our study is to challenge the generally accepted view that I_{Kur} is an atrial-specific current, and it has no physiological role in the ventricle.

The most important findings of the present study are the following:

(i) I_{Kur} inhibition shifts the plateau phase of the AP to the positive voltage direction and consequently shortens canine atrial APD.
(ii) I_{Kur} inhibition lengthens canine and human *ventricular* APs.
(iii) I_{Kur} inhibition prolongs canine PF APs.
(iv) Under the condition of impaired repolarization reserve I_{Kur} inhibition evokes EADs.

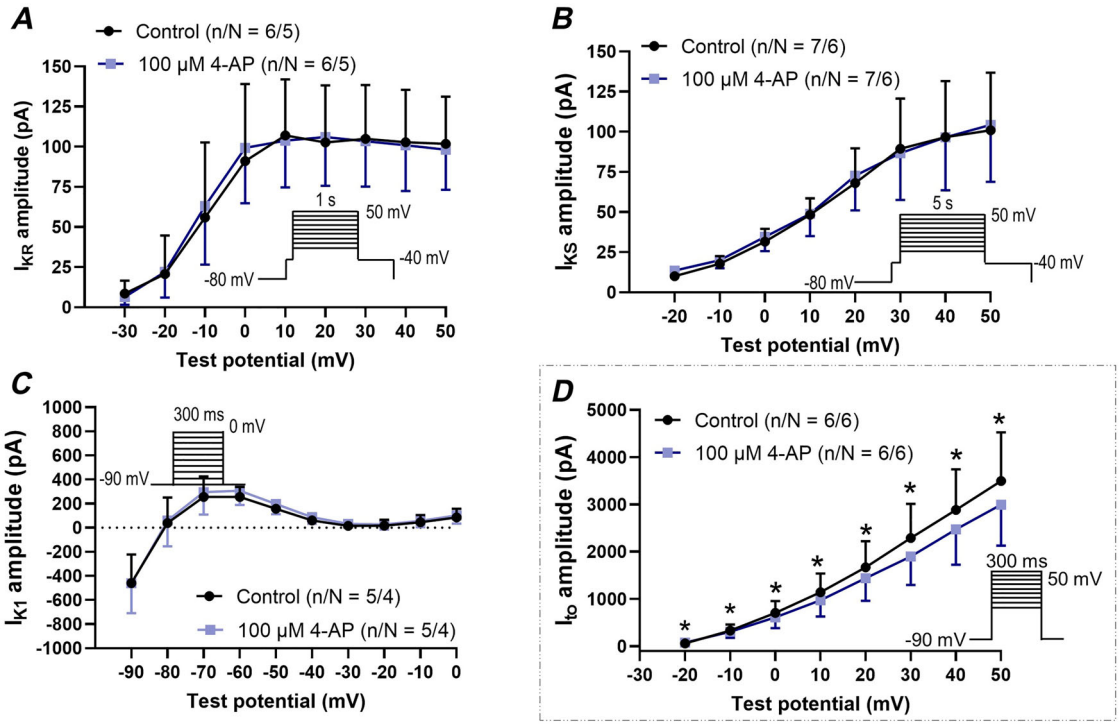

Figure 5. Selectivity measurements of 100 µM 4-AP application
Effect of 100 µM 4-AP on major repolarizing outward potassium currents, including (*A*) the rapid (I_{Kr}) and (*B*) slow (I_{Ks}) delayed rectifier potassium currents, (*C*) the inward rectifier (I_{K1}) potassium current and, lastly, (*D*) the transient outward potassium current (I_{to}). I_{Kr} and I_{Ks} currents have been identified and analysed as I_{Kr} and I_{Ks} tail currents, respectively. The four panels show current–voltage curves in control conditions and after application of 100 µM 4-AP. Insets indicate the voltage protocols. Data are expressed as means ± SD. *n/N* represents the number of myocytes (*n*) and the number of experimental animals (*N*).

Together these data indicate that I_{Kur} should be considered a substantial contributor to the repolarization reserve in ventricular myocardium, with a contribution comparable to I_{Ks}.

Comparison with previous studies

I_{Kur} is well characterized in canine and human atrial myocytes (Fedida et al., 1993; Fedida et al., 2003; Wang et al., 1993; Yue et al., 1996). At 35°C the current activates and deactivates rapidly in a voltage-dependent manner. Its activation time constant (τ) ranges from 3.5 to 0.33 ms between −10 and +60 mV, whereas deactivation occurs with a time constant of approximately 1 ms at −60 mV, and the current exhibits a relatively slow inactivation time (Yue et al., 1996).

In canine atrial myocytes I_{Kur} begins to activate at approximately −20 mV and reaches a peak amplitude of ∼1 nA at +60 mV (Yue et al., 1996). In the present study I_{Kur} recorded from canine ventricular myocytes was smaller, with an amplitude of approximately 90 pA at +50 mV, and was similar in magnitude and exhibited comparably rapid activation to that reported previously in canine ventricular myocytes by Sridhar et al. (Sridhar et al., 2007). Given the relatively small current amplitude

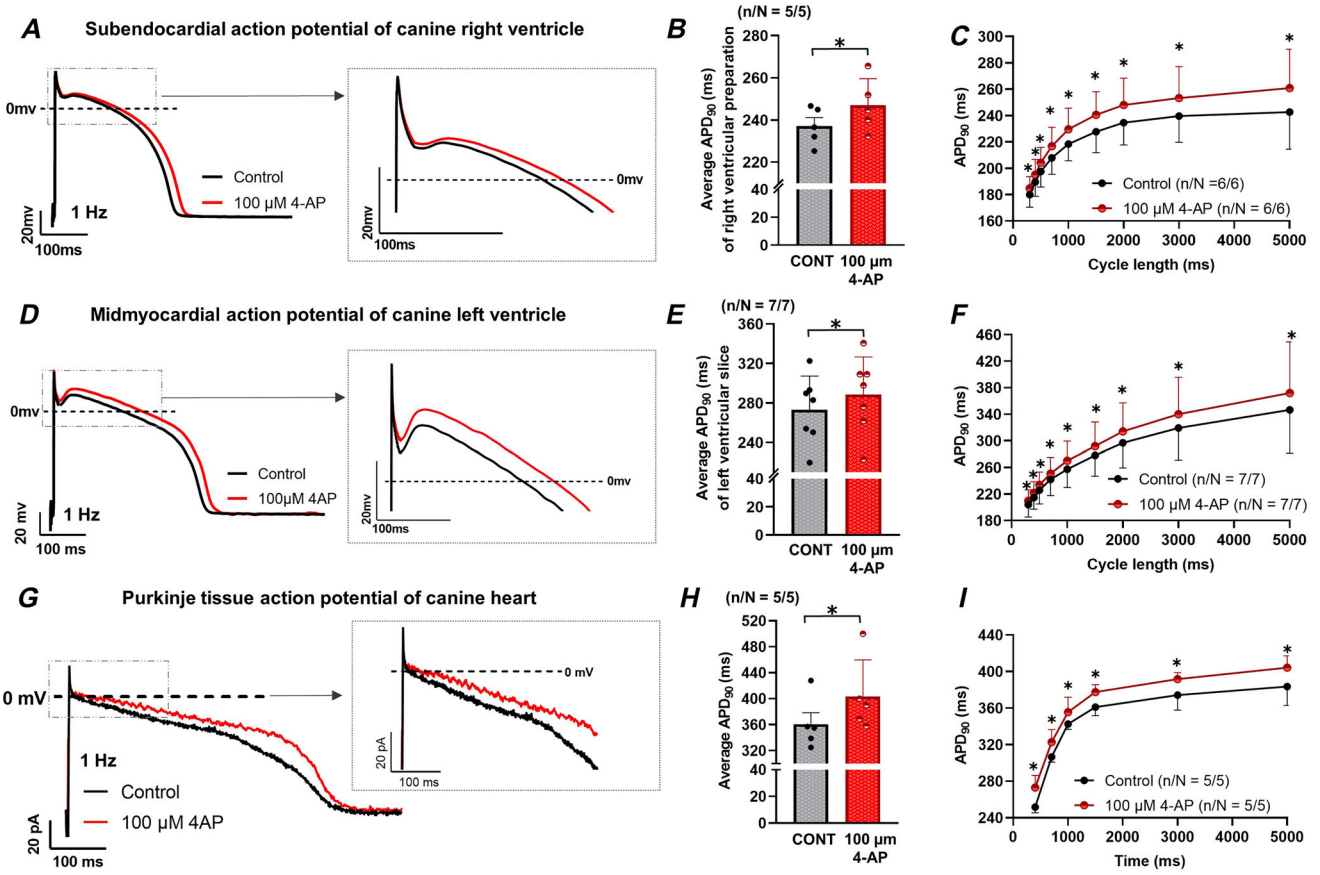

Figure 6. Action potential (AP) changes in different cardiac tissues following 100 μM 4-AP application
A, the representative recording shows the AP duration (APD) prolongation and plateau shift into more positive voltage range induced by 100 μM 4-AP in a subendocardial AP of canine right ventricle. *B*, the bar graph with the individual data shows the average APD_{90} prolongation of the right ventricular preparation at 1 Hz frequency in control conditions and after application of 100 μM 4-AP. *C*, the cycle length–dependent effect of 100 μM 4-AP on APD_{90} of right ventricular preparation. *D*, the representative recording shows the APD prolongation and plateau shift into more positive voltage range induced by 100 μM 4-AP in midmyocardial action potential of canine left ventricle. *E*, the bar graph with the individual data shows the average APD_{90} prolongation of the left ventricular slice at 1 Hz frequency in control conditions and after application of 100 μM 4-AP. *F*, the cycle length-dependent effect of 100 μM 4-AP on APD_{90} of left ventricular preparation. *G*, the representative recording shows the APD prolongation and plateau shift into more positive voltage range induced by 100 μM 4-AP in Purkinje tissue of the canine heart. *H*, the bar graph with the individual data shows the average APD_{90} prolongation of the Purkinje fibre at 1 Hz frequency in control conditions and after application of 100 μM 4-AP. *F*, the cycle length-dependent effect of 100 μM 4-AP on APD_{90} of the Purkinje fibre. Data are expressed as means ± SD. *n/N* represents the number of myocytes (*n*) and the number of experimental animals (*N*).

and its very rapid activation kinetics – consistent with the observations of Sridhar et al. – we did not attempt to further characterize its kinetic properties.

Impact of I_{Kur} inhibition on the cardiac AP

When comparing the impact of I_{Kur} on AP morphology in atrial and ventricular myocardium, it should be taken into account that atrial myocytes exhibit a plateau voltage in the range of −20 to 0 mV, as shown in Fig. 2 and observed in previous studies (Ford et al., 2013; Wang et al., 1993; Wettwer et al., 2004), whereas ventricular myocytes display a plateau at more positive potentials, typically between +20 and +40 mV.

Accordingly, the effective amplitude of I_{Kur} during the plateau phase can be estimated to be approximately 80–100 pA in atrial myocytes, whereas in ventricular myocytes it is in the range of 40–60 pA, that is not markedly different in magnitude.

In addition, in atrial myocardium during sinus rhythm a positive shift of the plateau voltage can lead to enhanced activation of $I_{Kr,}$ which may limit APD prolongation or can even result in APD shortening (Fedida et al., 2003; Ford et al., 2013; Wettwer et al., 2004). Because I_{Kr} is already fully activated at the ventricular plateau potential (from +30 to +40 mV) no or less effect is expected in the ventricle.

Consistent with this notion a moderate but significant APD prolongation was observed in ventricular myocardium in our experiments, with a magnitude comparable or even higher than that reported previously for I_{Ks} inhibition (Varro et al., 2000; Varro et al., 2021).

This APD prolongation was reverse rate dependent in all types of ventricular preparation; that is larger APD lengthening was observed as stimulation frequency was decreased. The consistent and marked reverse rate-dependent APD prolongation can be well explained by intrinsic properties of the APs in the sense that any APD lengthening is proportional to the length of itself,

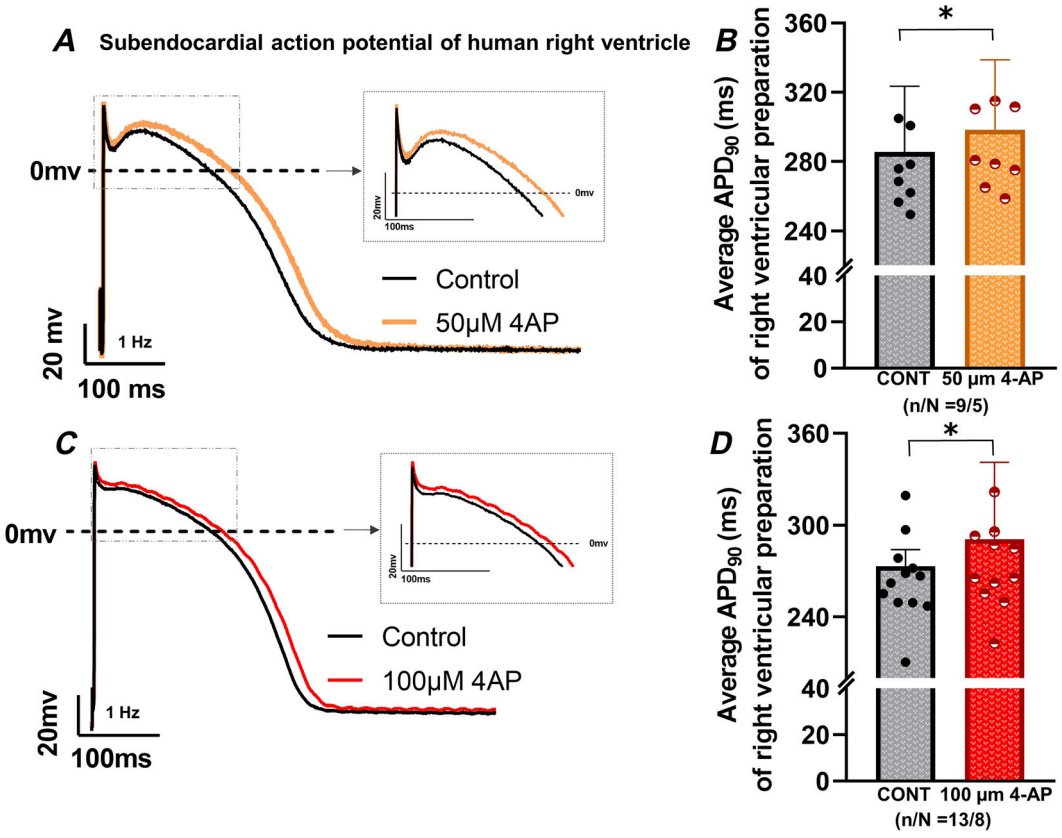

Figure 7. The effect of 4-AP in undiseased human cardiac preparations
A, the representative recording shows the action potential duration (APD) prolongation and plateau shift into more positive voltage range brought by 50 μM 4-AP. *B*, the bar graph with the individual data shows the average APD_{90} prolongation at 1 Hz frequency in control conditions and after application of 50 μM 4-AP. *C*, the original action potential recording. *D*, the bar graph show the effect of 100 μM 4-AP in human cardiac APD under the same experimental circumstances. Data are expressed as means ± SD. *n/N* represents the number of myocytes (*n*) and the number of donor hearts (*N*).

that is APD dependency of APD modulation (Bányász et al., 2009 and 2011; Bárándi et al., 2010 and Zaza, 2010). In addition, another possible mechanism cannot be excluded. The repolarization reserve theory was put forward by Dan Roden (1998, 2006) and experimentally demonstrated by our group reporting substantial (even/often leading to EAD) additive repolarization lengthening by combined 'I_{Kr} ±I_{Ks}', 'I_{Kr}±I_{K1}' and 'I_{Kr}±I_{to}' inhibitions (Varró et al., 2000; Biliczki et al., 2002; Jost et al., 2013; Virág et al., 2011).

In contrast to that observed in the ventricle, in the present study in atrial myocardium the effects of I_{Kur} inhibition resulted in shortening of the APD. In previous studies marked variability ranging from APD shortening to modest or pronounced prolongation had been reported (Fedida et al., 2003; Ford et al., 2013; Wang et al., 1993; Wettwer et al., 2004). This variability likely reflects differences in AP morphology and plateau voltage arising from species differences, regional heterogeneity within the atria or atrial electrical remodelling.

In PFs I_{Kur} inhibition also prolonged APD, on average to a greater extent than in ventricular tissue. This difference is consistent with the weaker repolarization reserve of PFs (reduced I_{Ks}) and their enhanced I_{NaL} activity.

Determination of I_{Kur}

It is sometimes difficult to fully distinguish I_{Kur} from I_{to}. In our experiments as previously reported by others we used two complementary approaches (Amos et al., 1996; Sridhar et al., 2007; Yue et al., 1996). First a +30 mV prepulse was applied to inactivate I_{to}, and selective I_{Kur}

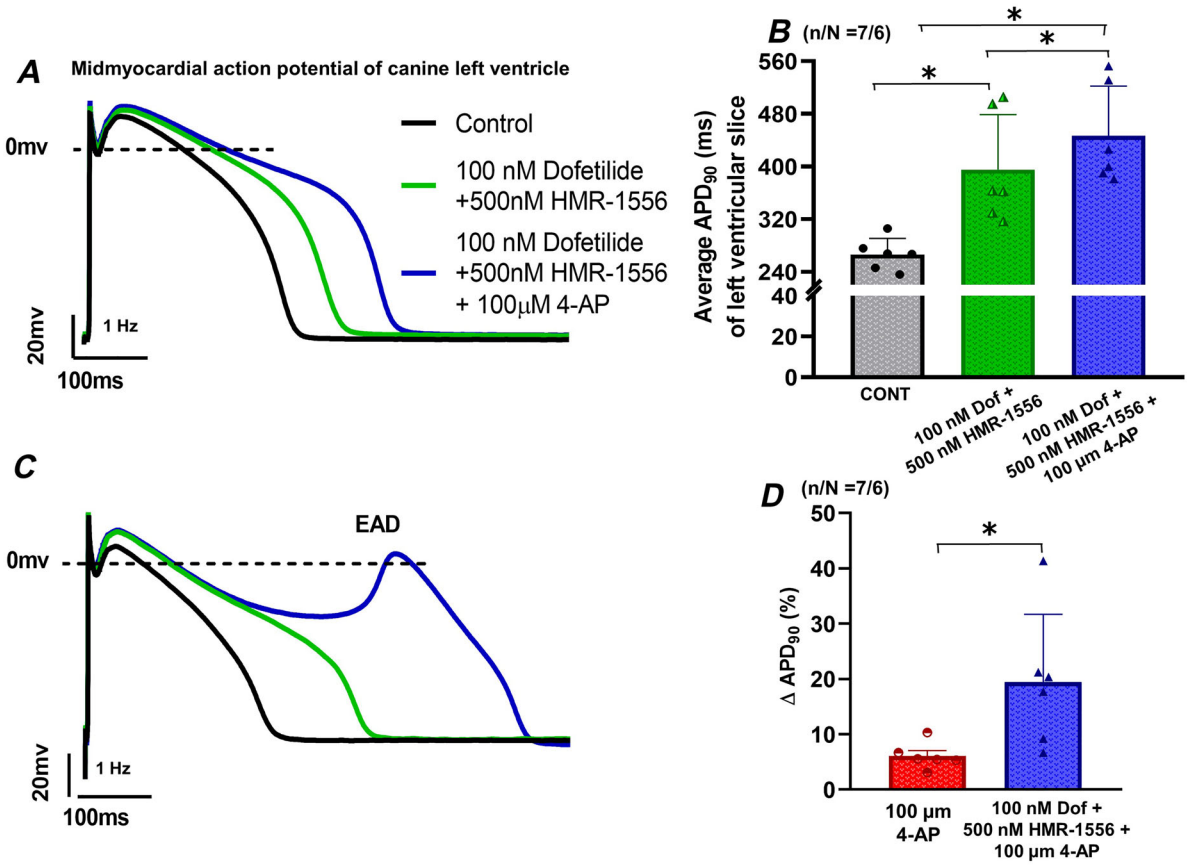

Figure 8. Effect of 100 μM 4-AP on left ventricular slices with impaired repolarization reserve
A, representative action potential recordings show the prolonging effect of 100 μM 4-AP (blue trace) in cardiac left ventricular slice with strongly attenuated repolarization reserve by I_{Kr} (100 nM dofetilide, DOF) and I_{Ks} (500 nM HMR-1556, HMR) blockers (green trace). Black trace represents the control measurement. *B*, the bar graphs with the individual data show the average APD_{90} changes in controls (black bar) and after the impaired repolarization-induced (DOF+HMR) average APD_{90} alone (green bar) and the combination of the DOF+HMR and 100 μM 4-AP (blue bar). *C*, the original action potential recording shows the observed 100 μM 4-AP–induced early afterdepolarization (EAD) after the attenuation of repolarization reserve in two out of five experiments. *D*, the bar graph shows the percentage increase in APD_{90} induced by 100 μM 4-AP alone (red bar, data originated from Fig. 6*E*) and by the combination of DOF+HMR and 100 μM 4-AP (blue bars). Data are expressed as means ± SD. *n*/*N* represents the number of myocytes (*n*) and the number of experimental animals (*N*).

inhibitor (4-AP) was then used to measure difference currents.

With respect to the selectivity of 100 μM 4-AP, we relied on previous studies demonstrating a favourable selectivity profile of 4-AP for I_{Kur} over I_{to} (Loose et al., 2014). In addition, we assessed the effects of 100 μM 4-AP on I_{to}, I_{Kr}, I_{Ks} and I_{K1} to exclude potential contributions of unintended inhibition of these currents to the observed AP changes. It was found that 100 μM 4-AP causes ∼15% decrease in I_{to} without effect on the further currents. Interestingly, the small decrease in I_{to} observed with 100 μM 4-AP in our experiments slightly differs from those reported by Wang et al. (Wang et al., 1993) and Sridhar et al., who did not observe the decreased amplitude of I_{to} after the application of 50 and 100 μM 4-AP (Sridhar et al., 2007; Sridhar et al., 2008). We do not think that this degree of I_{to} inhibition would cause a measurable APD prolongation. In addition it cannot be ruled out that the small I_{to} decrease in our experiments was due to the modest difference in series resistance (3.5 ± 1.2 to 6.4 ± 0.8 mΩ) between the two sets of experiments. Because fully selective I_{to} inhibitor is not available we also applied *in silico* modelling at 14% I_{to} inhibition (Fig. 9*C*), which indicated no discernible effect on APD.

I_{Kur} in PF

The magnitude of the 100 μM 4-AP-sensitive current measured in Purkinje cells does not differ significantly from that recorded in ventricular myocytes.

The only relevant published study regarding I_{Kur} was carried out in human cardiac Purkinje myocytes, and it showed small but statistically not significant 4-AP-sensitive sustained plateau current. In this study it was concluded that in PF unlike in the atria the existence of I_{Kur} is questionable (Han et al., 2002). The discrepancy of the results between this latter study and our present experiments can be due to differences in the experimental conditions and also due to the origin of the preparations, for example human *versus* canine.

Possible clinical implications

Based on our results in the ventricle because the prolongation of repolarization in both ventricular myocardium and PFs was modest, we do not suggest that I_{Kur} inhibition would exert a clinically useful class III antiarrhythmic effect, nor that it would significantly increase proarrhythmic risk under normal conditions.

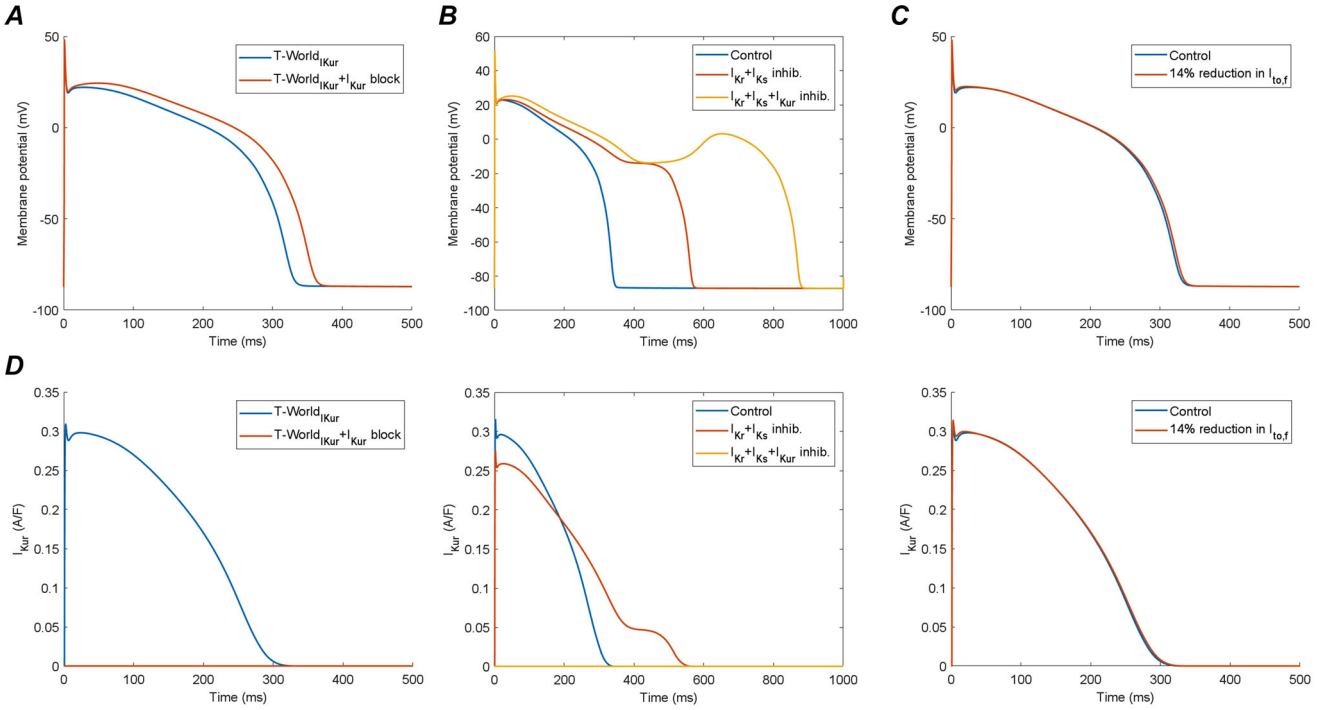

Figure 9. I_{Kur} inhibition prolongs the action potential duration (APD) and promotes early afterdepolarization (EAD) formation in *in silico* model of human ventricular action potential model
A, full blockade of I_{Kur} in T-World$_{IKur}$ prolongs the APD by 9.5%. *B*, an example of a cell within the studied population, which shows the control action potential, its prolongation by 66% inhibition of I_{Kr} and I_{Ks} and EAD formation following additional full inhibition of I_{Kur}. *C*, 14% inhibition of $I_{to,f}$ has negligible effect on the APD in T-World$_{IKur}$. *D*, I_{Kur} currents (left panel – I_{Kur} block alone; middle panel – I_{Kur} block after combined I_{Kr}+I_{Ks} inhibition; right panel – 14% of $I_{to,f}$ block effect) underlying the simulations shown in panels *A*, *B* and *C*, respectively.

This degree of APD prolongation does not necessarily result in clinically relevant QTc prolongation in healthy individuals, as has been shown in clinical investigations (Camm et al., 2019; Ford et al., 2013; Shunmugam et al., 2018). However, in preparations in which repolarization reserve was markedly attenuated by combined I_{Kr} and I_{Ks} inhibition we observed pronounced ventricular APD prolongation and occasional EADs.

The results of our *in silico* modelling regarding the I_{Kur} inhibition-evoked APD lengthening and EAD formations are in good agreement with the experimental findings. The slight discrepancies can be well explained by the methodological differences between the two approaches.

Therefore, in clinical situations where APD is lengthened or cardiac repolarization reserve is markedly impaired – due to congenital ion channel defects, factors or pathological settings such as heart failure, diabetes, hypokalaemia or exposure to repolarization-prolonging drugs – additional I_{Kur} inhibition may increase the risk of *Torsades de Pointes* ventricular arrhythmia and, consequently, sudden cardiac death. This potential risk should be recognized in clinical practice and carefully considered during future drug development.

Potential limitations

In the present study we investigated the possible effects of I_{Kur} current blockade in canine and human ventricular myocardium. However, we have investigated the effect of 4-AP blockade only in subendocardial, midmyocardial and PF tissues from canine and human hearts. It would be of interest in future studies to test the effect of I_{Kur} inhibition in subepicardial cells as well.

Conclusion

In conclusion we suggest that I_{Kur}, previously considered an atrial-specific current, exists and plays a significant fine-tuning role in both canine and human ventricles. In addition, I_{Kur} may importantly contribute to ventricular repolarization reserve, although its precise role remains to be investigated.

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Additional information

Data availability statement

The data generated and analysed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

Author contributions

I.K., L.V., A.V., I.B., N.J., L.T.: conception or design of the work. A.A.E.A., A.S.A.M., G.M., B.P., G.B., J.T., SD, T.A-L., V.D-H., I.K., M.N., L.V., N.N., N.J., Z.K., L.T.: acquisition, analysis or interpretation of data. A.A.E, A., A.S.A.M., G.M., B.P., G.B., J.T., SD, T.A-L., V.D-H., I.K., M.N., L.V., N.N., A.V., I.B., N.J., Z.K., L.T.: drafting the manuscript or critically revising it for important intellectual content. A.A.E.A., A.S.A.M., G.M., B.P., G.B., J.T., SD, T.A-L., V.D-H., I.K., M.N., L.V., N.N., A.V., I.B., N.J., Z.K., L.T.: final approval of the version to be published. A.A.E.A., A.S.A.M., G.M., B.P., G.B., J.T., SD, T.A-L., V.D-H., M.N., N.N., A.V., I.B., N.J., Z.K., L.T.: agreement to be accountable for all aspects of the work. The authors approved the final version of the manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported by the National Research Development and Innovation Office (NKFIH K-142738, K-147212, ADVANCED-150395, GINOP-2.3.2.-15-2016-0006 and GINOP-2.3.2.-15-2016-0047 to A.V., N.J. and I.B.; FK-142949 to N.N and Z.K.; STARTING-150884 to Z.K and TKP2021-EGA-32 to I.B.), the Ministry of Human Capacities Hungary (EFOP-3.6.2-16-2017-00006 to A.V., N.J. and I.B.), the Albert Szent-Györgyi Medical School institutional grant (SZTE AOK-KKA 2022 to N.J. and SZTE AOK-KKA 2024 to I.B.) and the Hungarian Research Network (HUN-REN TKI project to Zs.K., A.V., N.N. and N.J.). This study was also supported by Project RRF-2.3.1-21-2022-00003 'National Heart Laboratory, Hungary' implemented with the support provided by the European Union (to I.B. and B.M.) and the Pharmaceutical and Medical Device Developments Competence Centre of the Life

Sciences Cluster of the Centre of Excellence for Interdisciplinary Research, Development and Innovation of the University of Szeged, Hungary. The study was further supported by the Hungarian Academy of Sciences János Bolyai Scholarship (to Z.K.) and the Sir Henry Wellcome Fellowship (222781/Z/21/Z to J.T.).

Generative AI statement

We did not use AI either for writing the manuscript or during the review process.

Keywords

cardiac muscle, electrophysiology, I_{Kur} current, proarrhythmia, repolarization reserve

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History

Translational Perspective

The ultrarapid delayed rectifier potassium current (I_{Kur}) has long been considered an atrial-specific current with no functional role in the ventricles. In the present study we challenge this prevailing concept and investigate the potential role of I_{Kur} in ventricular repolarization. Our results clearly confirm the presence of I_{Kur} current in ventricular tissue using molecular cardiology, *in vitro* electrophysiological and *in silico* simulation techniques. We also report that I_{Kur} current has limited role in normal atrial repolarization but may contribute to ventricular repolarization reserve, although its precise role remains to be further investigated. Based on these observation we emphasize that these results certainly challenge the previously plausible hypothesis that selective I_{Kur} blockers could be developed as effective antiarrhythmic agents against atrial arrhythmias as AF/AFlu, without any possible proarrhythmic adverse effects in the ventricles.