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ARTICLE

Novel experimental results in human cardiac electrophysiology: measurement of the Purkinje fibre action potential from the undiseased human heart[1]

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Abstract: Data obtained from canine cardiac electrophysiology studies are often extrapolated to the human heart. However, ~~it was earlier~~ demonstrated that because of the lower density of its K^+ currents, the human ventricular action potential has a less extensive repolarization reserve. Since the relevance of canine data to the human heart has not yet been fully clarified, the aim of the present study was to determine for the first time the action potentials of undiseased human Purkinje fibres (PFs) and to compare them directly with those of dog PFs. All measurements were performed at 37 °C using the conventional microelectrode technique. At a stimulation rate of 1 Hz, the plateau potential of human PFs is more positive (8.0 ± 1.8 vs 8.6 ± 3.4 mV, $n = 7$), while the amplitude of the spike is less pronounced. The maximal rate of depolarization is significantly lower in human PKs than in canine PFs (406.7 ± 62 vs 643 ± 36 V/s, respectively, $n = 7$). We assume that the appreciable difference in the protein expression profiles of the 2 species may underlie these important disparities. Therefore, caution is advised when canine PF data are extrapolated to humans, and further experiments are required to investigate the characteristics of human PF repolarization and its possible role in arrhythmogenesis.

Key words: human, dog, heart, Purkinje fibre, ventricle, action potential, electrophysiology.

Résumé : Les données recueillies d'études en électrophysiologie cardiaque chez le chien sont souvent extrapolées à l'humain. Cependant, il a été démontré précédemment que, en conséquence de la densité plus faible de ses courants K^+, le potentiel d'action ventriculaire humain possède une réserve de repolarisation moins importante. Puisque la pertinence des données chez le chien au cœur humain n'a pas encore été pleinement clarifiée, le but de l'étude présente était d'établir pour la première fois les potentiels d'action des fibres de Purkinje (FP) humaines saines et de les comparer directement à ceux du chien. Toutes les mesures ont été réalisées à 37 °C à l'aide d'une méthode conventionnelle par microélectrode. À un taux de stimulation de 1 Hz, le potentiel plateau des FP humaines est davantage positif (8,0 ± 1,8 vs 8,6 ± 3,4 mV, $n = 7$), alors que l'amplitude de la pointe est moins prononcée. Le taux maximal de dépolarisation est significativement plus faible chez les FP humaines comparativement à celles du chien (406,7 ± 62 vs 643 ± 36 V/s, $n = 7$). Les auteurs assument que la différence appréciable des profiles d'expression protéique entre les deux espèces peut sous-tendre ces disparités importantes. La prudence est ainsi de mise lorsque des données obtenues dans les FP de chien sont extrapolées aux humains, et des expériences plus approfondies sont requises afin d'examiner les caractéristiques de la repolarisation des FP humaines et leur rôle possible dans l'arythmogenèse. [Traduit par la Rédaction]

Mots-clés : humain, chien, fibre de Purkinje, ventricule, potentiel d'action, électrophysiologie.

Introduction

The cardiac Purkinje fibre (PF) system plays an important role in impulse propagation and in the generation of cardiac arrhythmias (Aiello et al. 2002; Han et al. 2002). It seems to be particularly important in initiating transmural re-entry leading to torsades de pointes arrhythmias, often associated with long QT syndromes (Nattel et al. 2007). PFs may also play a role in the ventricular arrhythmias induced by delayed afterdepolarizations (DADs), intraventricular re-entry, and ventricular fibrillation.

Earlier electrophysiological studies demonstrated the PF action potentials from different species, describing their morphology and responses to pharmacological interventions (Attwell et al. 1979; Coraboeuf et al. 1979; Marban and Wier 1985; Varro et al. 1985*a*, 1985*b*; Noble 1986; Bril and Man 1989; Lathrop and Varro 1989; Campbell et al. 1991). In spite of these studies, the lack of published data on undiseased human PF action potentials means that only very limited information is available concerning the underlying transmembrane ionic currents and channel expressions.

From earlier examinations of the importance of the repolarization reserve in canine PFs (Roden 1998), we concluded that the role of the slow component of the delayed rectifier K^+ current (I_{Ks}) in the PF (and in the ventricular muscle) under normal conditions is minimal (Varro et al. 2000). However, in the event of an increased action potential duration (APD), the enhanced I_{Ks} reduces the proarrhythmic risk, providing an important safety factor in repolarization (Varro et al. 2000; Jost et al. 2005, 2007). Nagy et al. (2004) and Jost et al. (2013) demonstrated an important role for the sodium–calcium exchanger (NCX) in PF DADs, since the latter were completely eliminated by the application of NCX inhibitors:

Received 17 December 2014. Accepted 4 May 2015.

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[1]This article is part of a Special Issue entitled "Cardioprotection and Arrhythmias, Part 2."

SEA0400 and a novel compound ORM-10103. The first reported investigation of protein expression level in the canine heart (Han et al. 2002) demonstrated that the ion channel expression in the PFs, consistent with their diverging electrophysiological characteristics, differs considerably from that in the ventricular muscle. The first data on the ion channel protein expression in human PFs were presented by Gaborit et al. (2007). PF cellular electrophysiological data from the levels of expression of channel proteins to current analysis and basic pharmacological responses were reviewed by Boyden et al. (2010). The characteristics of the inward rectifier K^+ current (I_{K1}) and transient outward K^+ current (I_{to}) in Purkinje cells of the failing human heart were described by Han et al. (2002). The most important findings were that the I_{K1} densities in the PFs and ventricular cells were comparable, in the range of –110 to 0 mV, and that the I_{to} characteristics (i.e., recovery, 4-aminopyridine sensitivity) of human PFs were similar to those of canine PFs. The sustained 4-aminopyridine-sensitive current measured after I_{to} inactivation was larger in human than canine Purkinje cells. It was concluded that the sustained current in human Purkinje cells is a noninactivating or only slowly inactivating component of I_{to}.

It is generally believed that in cardiac electrophysiology, the dog serves as a reasonably good model for humans. Indeed, in accordance with this belief, the characteristics of the transmembrane ion channels (I_{Ks}, I_{to}, I_{K1}, and the rapid component of the delayed rectifier K^+ current (I_{Kr})) are comparable in canine and human ventricular myocytes (Varro et al. 1993; Wettwer et al. 1994; Magyar et al. 2000; Virag et al. 2001; Volders et al. 2003; Akar et al. 2004; Jost et al. 2005). However, a recent study led us to conclude that as a consequence of the smaller magnitudes of I_{K1} and I_{Ks}, the repolarization reserve in the human heart is significantly weaker (Jost et al. 2013), which results in important differences in drug responses between dogs and humans. Therefore, care must be taken in extrapolating canine data to humans, since the possibility that such discrepancies may also exist between canine and human PF action potentials cannot be ruled out. Thus, the aim of our present study was to provide direct experimental data on action potentials in undiseased human PFs and to compare these data with those for dog PFs.

Methods

The canine experiments were performed in compliance with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication No. 86-23, revised 1985). All canine experimental protocols were approved by the Ethical Committee for Protection of Animals in Research of the University of Szeged, Hungary (permit No. I-74-9/2009). The investigations performed on human cardiac samples conformed to the principles outlined in the Helsinki Declaration. All human experimental protocols were approved by the Regional and National Human Medical and Biological Research Ethics Committee, University of Szeged (permit No. 63/1997).

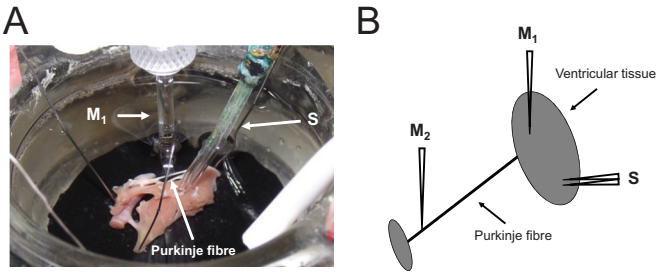
Human PF preparations

Undiseased hearts (n = 7) obtained from organ donors were explanted to obtain pulmonary and aortic valves for transplant surgery. Before cardiac explantation, the donors had not received medication other than furosemide, dobutamine, and plasma expanders. White free-running PFs with the attached ventricular tissue were excised from the right ventricle (Fig. 1). Similar preparations were obtained from the right ventricle of dogs (weighing 10–15 kg) previously anesthetized intravenously with 30 mg pentobarbital/kg.

Recording action potentials in multicellular PFs

Action potentials from PFs were recorded at 37 °C by using conventional microelectrode techniques. The preparations were mounted in a custom-made plexiglass chamber, allowing continuous superfusion with CO_2-saturated Krebs–Henseleit solution (containing 118.5 mmol/L NaCl, 4 mmol/L KCl, 1.2 mmol/L NaH_2PO_4, 25 mmol/L $NaHCO_3$, 1 mmol/L $MgSO_4$, 11 mmol/L glucose, 1.8 mmol/L $CaCl_2$; the pH was set to 7.4 by bubbling with CO_2), and stimulated with constant-current pulses of 1 ms duration at a rate of 1 Hz through a pair of bipolar platinum electrodes, using an electrostimulator (Hugo Sachs Elektronik, model 215/II). During the rate-dependent protocol, the preparations were stimulated by 10 pulses at each frequency. A complex, electrically coupled PF–ventricle preparation was used to perform experiments with 2 microelectrodes. In this case, the sample was stimulated from the ventricular side (Fig. 1). Sharp microelectrodes, with a tip resistance of 10–20 MΩ when filled with 3 mol/L KCl, were connected to an amplifier (Biologic Amplifier, model VF 102). The voltage output from the amplifier was sampled by using an AD converter (NI 6025, Unisip Ltd.). APDs determined at 90% and 50% levels of repolarization (APD_{90} and APD_{50}) were obtained by using the custom-made HSE-APES and Evokewave version 1.49 (Unisip Ltd.) software. Efforts were made to maintain the same impalement throughout each experiment.

Fig. 1. Human or dog Purkinje fibre (PF) preparation in the tissue bath (A), and a schematic diagram of the experimental arrangement of the measurement with one conventional electrode (A) and 2 electrodes (B). In the latter case, the ventricular part of the tissue was larger to provide sufficient area for electrode impalement. Abbreviations: S, stimulus electrode; M_1, microelectrode measuring the action potential from the ventricular tissue; M_2, microelectrode measuring the action potential from the PF.



Statistics

All values presented are arithmetic means ± SE. Statistical significance of differences was evaluated by using Student's t test for paired or unpaired data, as relevant. Differences were considered significant when the p value was <0.05.

The beat-to-beat variabilities (BVRs) of APD_{90} and APD_{50} were calculated by the analysis of 40 consecutive action potentials from the steady-state sections, using the following formulas:

$$BVR_{APD90} = \sum \frac{(APD_{90};\, i+1 - APD_{90};\, i)}{(n_{beats} x \sqrt{2})}$$

$$BVR_{APD50} = \sum \frac{(APD_{50};\, i+1 - APD_{50};\, i)}{(n_{beats} x \sqrt{2})}$$

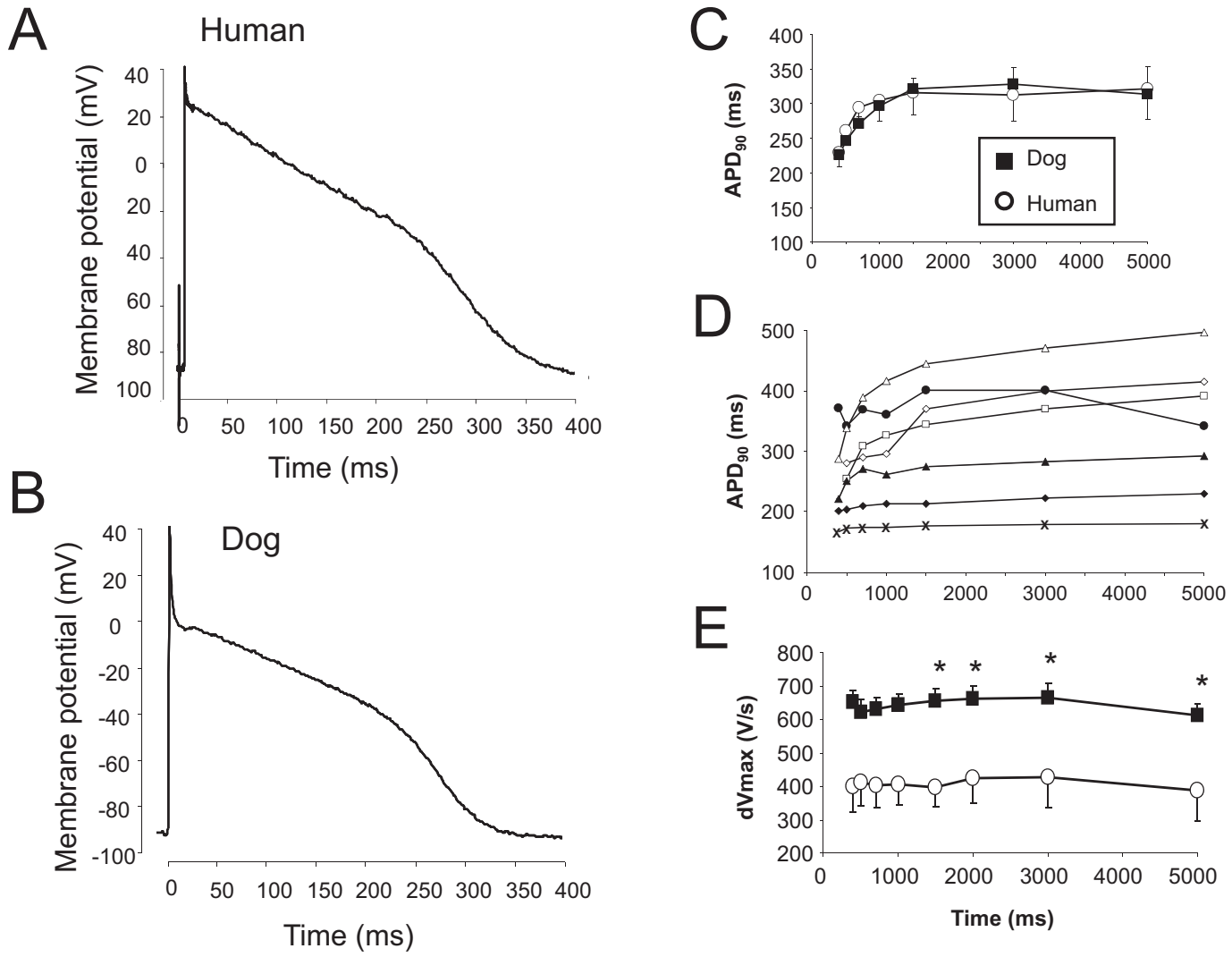
Since we compare data both within and between species, the statistical analysis of BVR was performed by one-way ANOVA.

Results

Frequency-dependent behavior of PF APD_{90} and V_{max}

The frequency-dependent changes in APD_{90} and V_{max} were measured by applying a stimulus pattern from 400 to 5000 ms cycle length in human (Fig. 2A) and canine (Fig. 2B) PFs. The frequency-dependent changes in APD_{90} were identical in the 2 types of PFs (Fig. 2C). In the individual human experiments (Fig. 2D), the rate dependence reveals considerable variation in the APD values at

Fig. 2. Comparison of characteristics of human (A) and dog (B) action potentials. The spike-and-dome configuration is pronounced in the dog but less so in the human case. Further, the human plateau potential, unlike that in the dog, is in the positive voltage range. The right side of the figure shows the corresponding mean of the frequency-dependent action potential duration (APD_{90}) values (C), the rate-dependent behavior of individual human preparations (D), and the V_{max} values (E) (data are means ± SE, $p < 0.05$, $n = 7$ from 7 hearts).



1000 ms, whereas relatively similar frequency-dependent behavior was observed.

The frequency-dependent characteristic of V_{max} was also similar in both cases, except that the depolarization rate was significantly lower in humans than in canines (Fig. 2E).

Analysis of PF phase 1 repolarization

The PF action potential was measured from right ventricular free-running PFs of both dogs and humans. The main difference was the lack of a pronounced phase 1 repolarization (action potential spike) for humans, and the plateau level was therefore higher. Moreover, the action potential spike amplitude was markedly smaller than that for dogs (Fig. 3) (9.8 ± 3.4 mV for humans vs 34.2 ± 2.8 mV for dogs, $p < 0.05$, $n = 7$). The slope decay of the action potential spike was estimated by using standard exponential fitting with 1 term, but we did not find a significant difference between the tau values of the 2 species (1.77 ± 0.42 ms for humans vs 2.1 ± 0.17 ms for dogs, $n = 7$).

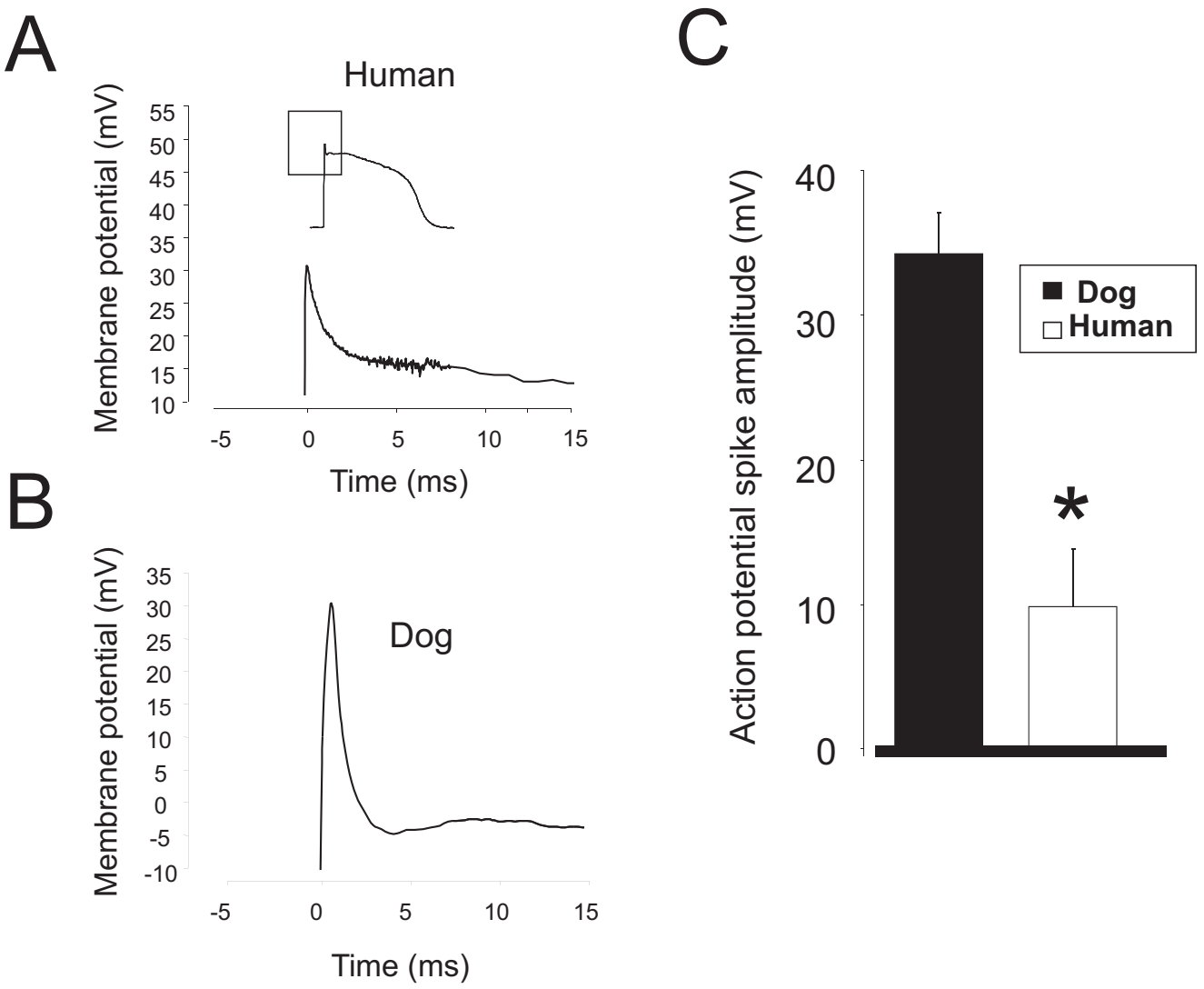
Analysis of the PF plateau level

As a possible consequence of the previous result, the plateau characteristic of the action potential in human PFs was significantly different from that in canine PFs. The mid-plateau voltage level (Fig. 4), which was measured at the half-time of APD_{90}, was in the positive voltage range (8.0 ± 1.8 mV) for humans, but for dogs it was in the negative voltage range (–8.6 ± 3.4 mV, $p < 0.05$, $n = 7$). The slope measured as the time derivative of the plateau voltage was steeper for humans than for dogs (–0.195 ± 0.2 mV/ms for humans vs –0.09 ± 0.01 mV/ms for dogs, $p < 0.05$, $n = 7$; Fig. 4).

Parallel measurements of ventricular and PF action potentials

Simultaneous measurements of the ventricular and PF action potentials were carried out by the technique with 2 conventional microelectrodes to assess the APD_{90} dispersion between the 2 adjacent regions. The PF–ventricular dispersion was calculated by subtracting the PF APD_{90} from the respective ventricular APD_{90}. It proved to be 128 ± 5.1 ms ($n = 7$) for dogs vs 59 ± 20 ms ($n = 2$) for humans at the same stimulation frequency of 1 Hz. Although the number of observations for human PF preparations is low, which is an obvious limitation, the data appear to suggest a lower dispersion rate in humans, which could be a consequence of the

Fig. 3. A comparison of human (A) and dog (B) spike morphology and amplitude. The magnitude of the action potential spike is more than 2-fold higher in dogs, as illustrated in panel C. The amplitudes of the spikes were calculated from the maximal point of action potential upstroke to the end-point of phase 1 repolarization (data are means ± SE, $p < 0.05$, $n = 7$ from 7 hearts).



F5 "ventricular-like" shape of the human PF action potential (Figs. 5A and 5B).

Analysis of BVRs of APD_{90} and APD_{50}

The short-term variability of APD was calculated by the analysis of 40 consecutive action potentials applying the formulas described in the Statistics section of Methods. The variability in a F6 representative experiment was depicted in a Poincaré plot (Figs. 6A and 6B), where each APD value was plotted against the previous action potential. Within a species, we did not find a significant difference between the variabilities of APD_{90} and APD_{50} (1.12 ± 0.16 and 0.87 ± 0.21 ms, respectively, for humans; 2.09 ± 0.69 and 2.5 ± 0.68 ms, respectively, for dogs; one-way ANOVA). At the same time, we observed a significantly lower BVR of APD_{50} for humans than for dogs (Fig. 6C).

Discussion

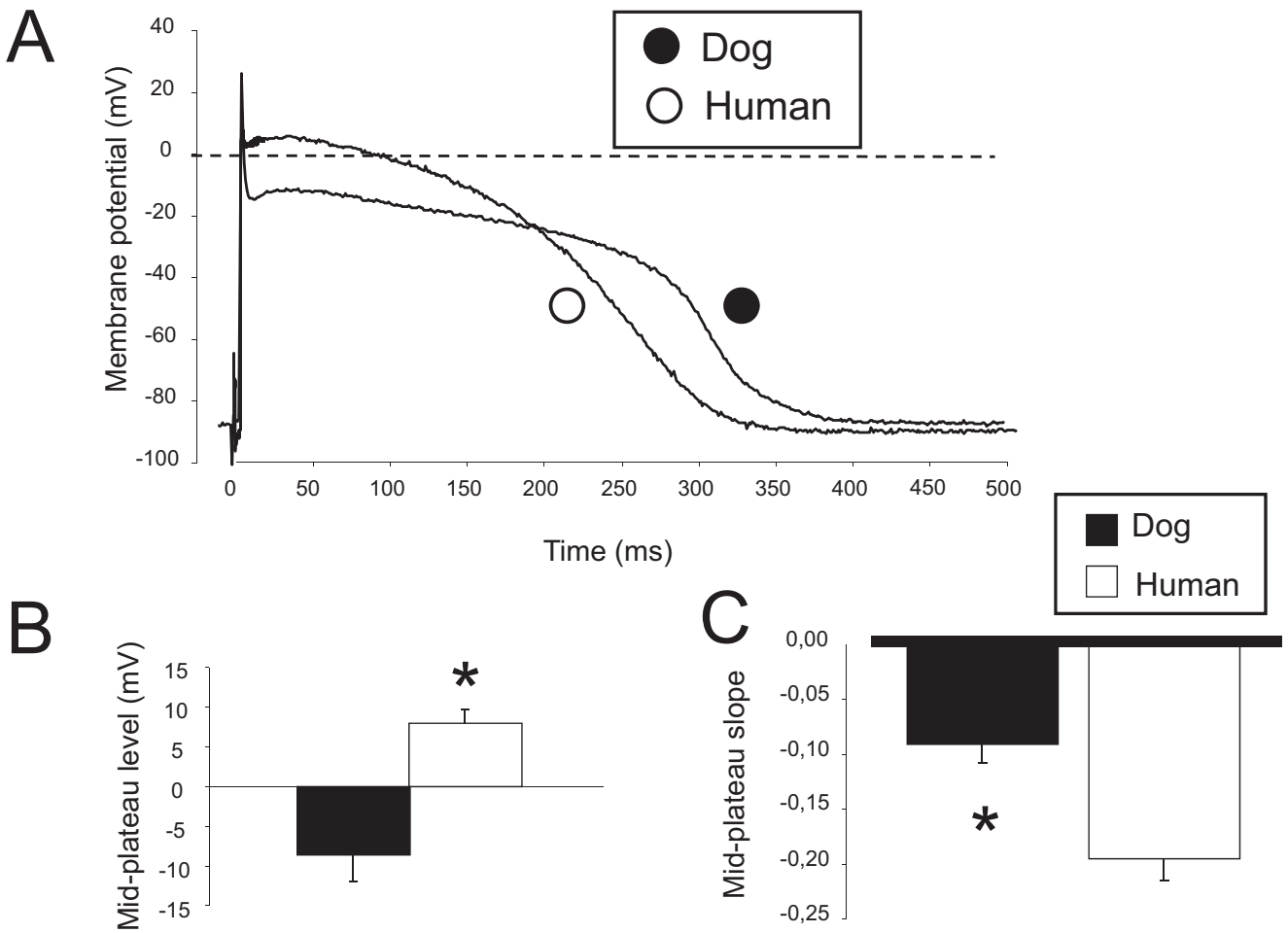
The goal of this study was to evaluate the characteristics of the human Purkinje action potentials recorded from free-running PFs obtained from undiseased donor hearts and to compare them with the corresponding canine data. We are not aware of any previous study of the action potential of undiseased human PFs in intact tissue.

Our major finding is that the shape of the PF action potential for humans is more "ventricular-like" than that for dogs, with the former having a smaller spike amplitude and a less steep plateau phase, which is in a more positive membrane potential range in humans than in dogs. We speculate that this special characteristic of the human PF action potential may be a consequence of the differing channel protein composition and a function of the Purkinje cells and (or) human PFs containing ventricular cells, which influence the PFs electrotonically. To explore this issue, further morphological and (or) single-cell experiments are required.

What are the consequences of the shape of the human PF action potential?

Several studies have led to the claim that the dispersion rate between the ventricular and PF APDs could be harmful if K^+ channel inhibitors lengthen the ventricular and PF action potentials to different extents, with the result that a potentially arrhythmogenic dispersion can build up (Antzelevitch 2008). Furthermore, the effect of an inherited genetic mutation in a channel protein

Fig. 4. Analysis of the action potential plateau phases, with calculation of the mid-plateau level at the half-point of action potential duration (APD_{90}), and the plateau slope measured as the time derivative of the plateau voltage (A). In all measurements, the Purkinje fibre (PF) plateau for humans reached a significantly higher potential than that for dogs (B). For humans the plateau has a steeper voltage (C), so that its derivative was higher than that calculated for dogs. Data are means ± SE, $p < 0.05$, $n = 7$ from 7 hearts.



(e.g., LQT mutations), which has a negligible impact on the action potential under basic conditions, could be augmented under a K^+ channel blockade, especially during slow pacing. It may be feasible to assume that the somewhat enhanced ventricular-type characteristics of the human PF action potential could result in a lower dispersion rate between the ventricle and the PFs in humans compared with dogs.

A further possible consequence of the shape of the human PF action potential could be an increased repolarization reserve, since the higher mid-potential of the plateau level may increase the activation of both I_{Kr} and I_{Ks}, leading to their enhanced contribution to the repolarization process.

Possible consequences of the shape of the PF action potential on the repolarization reserve

We previously compared human and dog ventricular repolarization reserves and found a lower repolarization capacity in humans due to the reduced activities of I_{K1} and I_{Ks} (Jost et al. 2013). This causes a greater level of response of the ventricular action potential in humans than in dogs to the same pharmacological intervention.

The repolarization reserves of human and canine PFs may display both similarities and differences. We suggest that I_{K1} is weaker in human PFs than in canine PFs (Gaborit et al. 2007) and, therefore, attenuates the PF repolarization capacity. At the same time, the higher plateau level of the human PF action potential may also influence the kinetics of the K^+ currents, i.e., the elevated activating potential may generate increased current amplitudes, which may exert the opposite effect on the extent of repolarization.

Interestingly, the I_{K1} density in the PFs in the failing human heart has been reported to be comparable with that in the ventricle (Han et al. 2002), while I_{K1} in the PFs in the undiseased heart was about one-third in magnitude of that in the ventricle (Gaborit et al. 2007). The question arises of whether, in spite of the well-known I_{K1} downregulation in ventricular remodeling, I_{K1} in the PFs is upregulated in heart failure (Nattel et al. 2007).

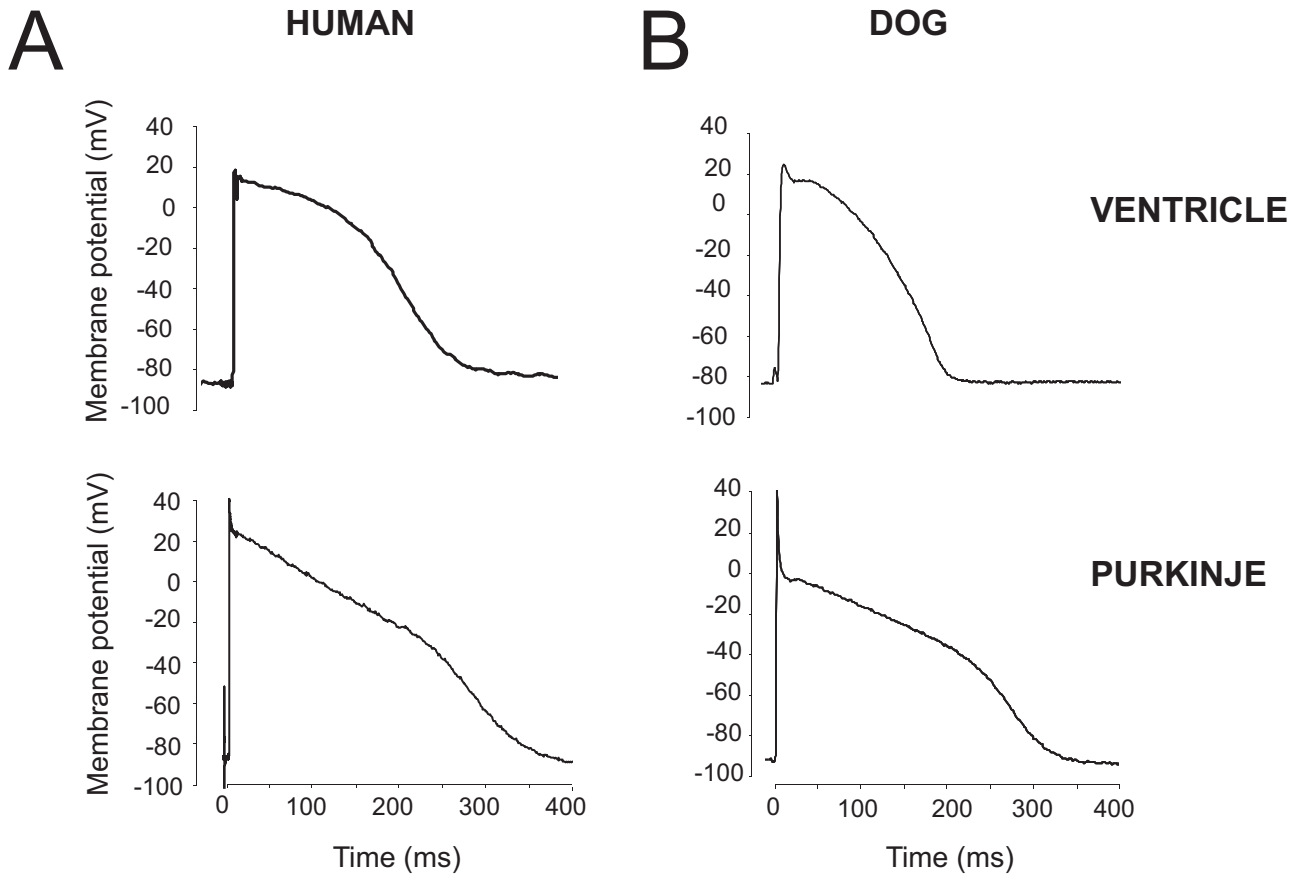
Comparison of human and canine Purkinje action potentials

We set out to compare human and canine Purkinje action potentials, but since data are not available on ionic currents in human PFs, we decided to compare protein expression levels in the 2 species.

Sodium current

The Purkinje action potentials display a very fast upstroke in phase 0. In dogs, the underlying current is carried by the Nav1.5 current, which demonstrates relatively low tetrodotoxin (TTX) sensitivity (Gintant et al. 1984). Since both the ventricular and PF action potentials in dogs are abbreviated by a low concentration of TTX, the noncardiac, TTX-sensitive channels have also been

Fig. 5. Parallel measurements of ventricular (up) and Purkinje (down) action potentials with 2 microelectrodes to analyze the actin potential duration (APD_{90}) dispersion in humans (A) and dogs (B). Although there were only 2 human measurements, it is apparent that the dispersion was lower for humans (n = 7 from 7 dog hearts, n = 2 from 2 human hearts).



suggested to contribute to sodium current in both preparations. While the canine ventricle displays neuronal Na^+-channel isoforms Nav1.2, Nav1.3, and Nav1.6, the PFs express Nav1.1 and Nav1.2, both noncardiac isoforms (Boyden et al. 2010). Human PFs express Nav1.5 at a level similar to that expressed in dog PFs (Han et al. 2002; Gaborit et al. 2007); accordingly, the observed action potential amplitudes were identical in the 2 species, though the rate of depolarization was found higher in dogs (Fig. 2E). The underlying mechanism could involve different Na^+-channel isoforms and (or) kinetics in humans. However, if human PFs contain electrotonically coupled ventricular cells, the rate of action potential upstroke could be markedly reduced via the considerably slower kinetics of ventricular depolarization.

Ca^{2+} handling

In humans, the levels of the investigated Ca^{2+}-handling proteins NCX1, SERCA2, CASQ2, and RYR2 were previously found to be lower in the PFs than in the ventricle (Gaborit et al. 2007). These reduced expression levels were suggested to be related to the lower contractile ability of the PFs. Similarly, L-type Ca^{2+} current in the canine PFs was markedly reduced, relative to that in the ventricle, whereas the level of T-type Ca^{2+} current expression was nearly identical to that of L-type Ca^{2+} current. The lower level of NCX1 expression in the dog was assumed to be responsible for the increased digitalis sensitivity of the canine PFs (Han et al. 2002). In line with this, it was demonstrated that NCX inhibition can effectively decrease the DADs in the canine PFs, suggesting an important role for NCX in arrhythmogenesis (Nagy et al. 2004; Jost et al. 2013). Unfortunately, similar data from human PF preparations are not available.

Transient outward current

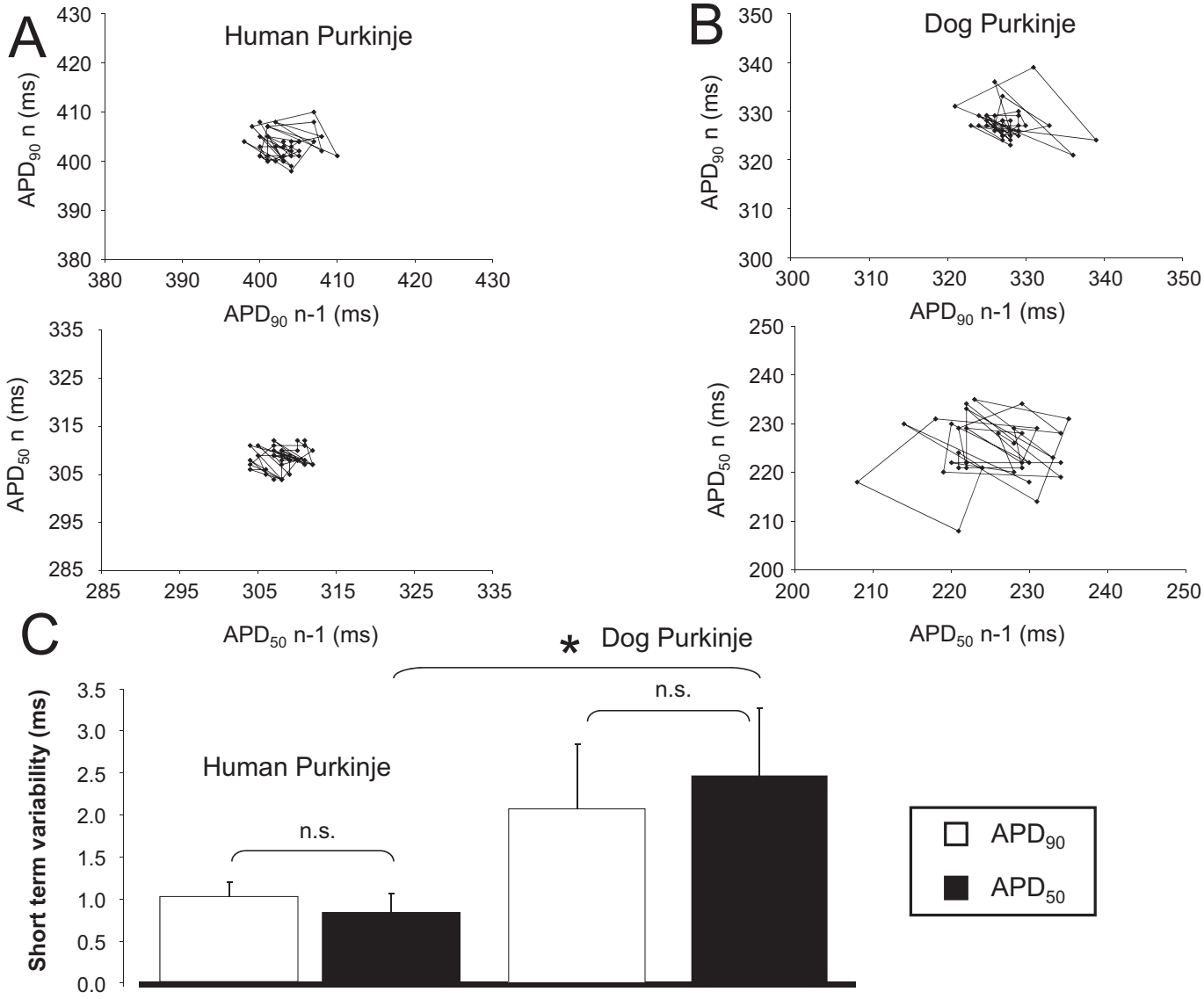
Like in the human ventricle, I_{to} in human PF cells is primarily generated by the voltage-gated K^+ (Kv) channels 4.3, 1.4, 1.5, and 3.4. Furthermore, the expression of the Kv4.3 subunit has been found to be abundant, while the expression of KChIP2 (Kv channel-interacting protein 2) proved to be low in both human and canine Purkinje cells (Gaborit et al. 2007). This result was consistent with the finding of Han et al. (2002), who found a very slow recovery of canine I_{to} from inactivation. Conversely, KChAP (Kv channel-associated protein) and DP6 exhibited high expression in the Purkinje cells (Kuryshev et al. 2001; Xiao et al. 2013). These proteins seem to play no physiological role in the ventricular tissue. In contrast, Xiao et al. (2013) assume that KChAP may play a physiological role in the Purkinje I_{to}.

Despite the similarities in the expression profiles of the Ca^{2+}-handling proteins and the channel and accessory proteins of I_{to}, marked differences were found between the 2 species in spike-and-dome morphology. This discrepancy may be underlined by differences in the Ca^{2+} current and (or) the electrophysiological characteristic of I_{to}. Since there is a marked difference between the Purkinje and ventricle spike amplitudes in dogs, the question again arises of whether the small spike observed in human PFs could be a consequence of electrotonically coupled ventricular cells in these PFs.

Delayed rectifier currents

The demonstration of delayed rectifier currents in single Purkinje cells is difficult because of the sensitivity of I_K to cell isolation, but I_{Kr} and I_{Ks} data from human PF preparations are not available. Nonetheless, there is an interesting difference between

Fig. 6. Analysis of the beat-to-beat variabilities of action potential duration (APD_{90}) and APD_{50}. Panels A and B depict the APD_{90} (upper panels) and APD_{50} (lower panels) variabilities collected from 40 consecutive action potentials by using Poincaré plots ~~in~~ humans (A) and ~~in~~ dogs (B). Statistical analysis did not reveal a significant difference between the APD values (C) within the same species, but there was a significant difference in APD_{50} variability between the 2 species. Data are means ± SE, $p < 0.05$, n = 7–7 from 7 hearts in each species.



humans and dogs in the levels of expression of proteins related to the delayed rectifiers. In dogs, the levels of the ERG, KvLQT1, and MinK proteins were significantly lower in the PFs than in the ventricle, which may explain the lower magnitude of I_{Kr} and I_{Ks} and may contribute to the longer APD_{90} in the PFs. However, in humans, where the APD in the PFs is also longer than in the ventricle, the expression of the levels of HERG and KvLQT1 proteins were found to be similar to those in the ventricle (Han et al. 2002; Gaborit et al. 2007), suggesting that additional mechanisms may contribute to the longer APD. A promising candidate could be I_{K1}, the level of expression of which has been found to be lower than that in the ventricle (Gaborit et al. 2007).

At the same time, the data obtained from protein expression analysis may imply the enhanced function of I_{Kr} and I_{Ks} in human PFs, which may increase the repolarization reserve and might explain the lower APD_{50} variability of human PFs as compared with canine PFs (see Fig. 6).

Inward rectifiers

Inward rectifiers, including I_{K1} (Kir2.1, Kir2.2, and Kir2.3), $I_{K(ATP)}$ (Kir6.1 and Kir6.2), and $I_{K(Ach)}$ (Kir3.1 and Kir3.4) carry K^+ currents, which have important roles in the final phase of the repolarization and in setting the level of the resting membrane potential. The level of expression of Kir2.x in human PFs has been reported to be lower than that in the ventricle (Gaborit et al. 2007). We previously demonstrated a lower repolarization reserve in human ventricle than in canine ventricle (Jost et al. 2013)~~,~~ and concluded that this mechanism is due in part to the reduced level of expression of I_{K1} proteins in humans. Since human PFs express a lower level of Kir2.x than that in the ventricle, it may attenuate the repolarization capacity of human PFs and may enhance the beat-to-beat APD variability. However, the observed resting membrane potential (–85.03 ± 1.9 mV) does not indicate a marked reduction in I_{K1}. In line with this, the temporal variability of APD_{90} was lower in human PFs, though the differences were insignificant and remained within the experimental variance (Fig. 6).

Conclusions and future perspectives

To the best of our knowledge, this is the first study to provide AP data on undiseased human PFs. Although human and canine PFs exhibit numerous similarities in protein expression profiles, we observed less "typical" Purkinje action potentials in humans. These action potentials proved to be more similar to the ventricular waveforms by having their plateau level in the positive membrane potential range and displaying much reduced spike-and-dome morphology. The waveform differences may have a role in the function of the human PF repolarization reserve and, therefore, may influence the kinetics of various transmembrane ionic currents, drug responses, and APD dispersion in several cardiac diseases. Hence, it is important that care be taken in extrapolating to humans data obtained on canine PFs, and further studies are required with the aim of current analysis and determination of the basic pharmacological properties of human PFs to achieve a better understanding of the pathomechanisms of human arrhythmias and to promote future antiarrhythmic drug development.

Acknowledgements

This work was supported by the Postdoctoral Program of the Hungarian Academy of Sciences (for N.N.) and by the European Union and the State of Hungary and was co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 "National Excellence Program". Grants were received from the Hungarian Research Fund OTKA (NN-109904, ANN113273, and NK-104331), the National Development Agency, and co-financed by the European Regional Fund (TÁMOP-4.2.2A-11/1/KONV-2012-0073 and TÁMOP-4.2.2.A-11/1/KONV-2012-0060) and the Hungarian Academy of Sciences. The authors declare they have no conflicts of interest.

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