Final report NKFIH PD116011

Pages 1-19

1/ During our cardiac electrophysiological investigations, in Purkinje fiber models (pinacidil and acetylcholine) of Early Repolarization Syndrome we found that 5 μM acetylcholine significantly lengthened the action potential duration (APD) in Purkinje fibers when it was administered after 5 μM pinacidil application.

We further analysed these observations in details, resulting in the scientific paper (accepted for publication) entitled:

Muscarinic agonists inhibit the ATP-dependent potassium current and suppress the ventricle-Purkinje action potential dispersion

containing the following results below. For more details, please see the uploaded article at:

http://real.mtak.hu/116123/

1. Acetylcholine lengthened the APD after pinacidil-mediated action potential shortening

Canine Purkinje fibers and ventricular papillary muscles were paced at 500 ms cycle length. In canine Purkinje fibers (PFs; n=15), acetylcholine (5 μM) did not affect the repolarization (233.6±4.7 to 231.7±4.6; Figures 1A and 1E). In contrast, in canine Purkinje fibers (n=8), the IK-ATP activator pinacidil, applied in 5 μM concentration, significantly abbreviated APD90 (207.7±7.0 ms vs 113.1±9.1 ms, $p<0.05$) values. After steady state was reached, acetylcholine was administered. Within 3 minutes, acetylcholine prolonged APD90 to 147.3±7.4 ms, partially reversing the effects of pinacidil (Figures 1B and 1E; $p<0.05$).

Similarly, as observed in Purkinje fibers, 5 μM acetylcholine alone failed to influence the APD of the ventricular muscle (APD90: 172.6±5.7 ms vs 172.8±5.3 ms). Pinacidil (n=5; 5 μM) pretreatment significantly abbreviated the APD90 value (187.9±4.5 ms vs 163.7±6.4 ms, $p<0.05$), similarly to the effects observed in the case of PFs. After a period of 30 minutes, sufficient to reach a steady state, acetylcholine was added to the superfusate. Within 4 minutes, acetylcholine (5 μM) prolonged APD90 to 172.1±7.4 ms ($p<0.05$), thus partially reversing the effects of pinacidil (Figures 1D and 1E).

2. Acetylcholine decreased the calculated APD dispersion between PF and VM

The changes in the difference between the APD90 values of PF and VM can be used to infer the effects of pinacidil and acetylcholine on the dispersion between these cardiac tissue types (Figure 2). The control APD90 dispersion (9.5%, 20 ms) was significantly increased upon 5 μM pinacidil application (44.7%, 51 ms). On the other hand, subsequently applied 5 μM acetylcholine markedly decreased the repolarization heterogeneity (16.9%, 28 ms; $p<0.05$).

3. Carbachol decreased the pinacidil-induced current activation

During ionic current measurements, voltage ramps were used from a holding potential of 90 mV. Membrane potential was hyperpolarized to 120 mV, and then was slowly (over 36 s) depolarized to 60

mV. Ionic currents were analyzed and compared at 0 and +30 mV. We found that carbachol did not change the control current when it was applied without pinacidil (0 mV - control: 0.20±0.2 pA/pF vs 3 µM carbachol: 0.32±0.2 pA/pF, n=6 and +30 mV - control: 0.55±0.4 pA/pF vs 3 µM carbachol: 0.74±0.3 pA/pF, n=6). In contrast, when 5 µM pinacidil was applied first, subsequently employed carbachol significantly reduced the current at both voltages (0 mV – control: 0.24±0.2 pA/pF → 5 µM pinacidil: 2.03±0.3 pA/pF → 3 µM carbachol: 1.51±0.4 pA/pF, n=8, $p<0.05$. +30 mV - control: 0.78±0.6 pA/pF → 5 µM pinacidil: 3.17±0.3 pA/pF → 3 µM carbachol: 2.26±0.3 pA/pF, n=8, $p<0.05$).

These measurements were carried out with acetylcholine as well. However, we found carbachol to be more stable during the applied long voltage protocol.

4. Acetylcholine restored the APD after hypoxia-induced action potential shortening

Simulated hypoxia, achieved by gassing the solution with N2 and CO2 instead of O2 and CO2, resulted in a significant abbreviation of APD90 from 181.4±5.7 ms to 135.0±8.6 ms ($p<0.05$, Figures 4A and 4B), and a decrease in amplitude (103.7±2.8 mV vs 92±3.5 mV). The maximum rate of depolarization was also decreased (185.8±15.8 V/s vs 156.1±20.6 V/s). When applied during hypoxia, 5 µM acetylcholine caused a significant APD90 prolongation to 164.4±4.4 ms, partially reversing the effect of hypoxia on the repolarization. AMP returned to a normal range (102.1±1.6 mV), while Vmax remained at 156.0±16.1 V/s.

5. Acetylcholine caused a slight abbreviation in human Purkinje fibers

In human PFs (n=2), acetylcholine in 5 µM concentration caused a slight abbreviation of APD90 from 269.0±28.4 to 251.6±42.85 ms and APD50 from 184.4±20.0 ms to 173.3±27.1 ms without affecting other characteristics of the action potential (Figure 5).

Discussion

In this study we investigated the electrophysiological effects of muscarinic agonists on the IK ATP current. We found that (i) under normal conditions acetylcholine did not influence the action potential duration. (ii) In contrast, when IK-ATP was pharmacologically activated by pinacidil, subsequently applied acetylcholine lengthened the action potential duration as well as (iii) reduced the pinacidil-induced ventricle-Purkinje APD dispersion. (iv) In line with this, carbachol inhibited the IK ATP that was previously activated by pinacidil. (v) Acetylcholine increased the APD after hypoxia-induced action potential shortening.

Acetylcholine inhibits the IK-ATP in canine ventricular myocytes

It is well known that acetylcholine shortens the atrial APD and has been implicated in atrial fibrillation (Nakayama et al, 1968). Acetylcholine directly affects the GIRK1/4 or Kir3.1/Kir3.4 channels (Nobles et al, 2018; Corey and Clapham, 1998), encoded by KCNJ3 and KCNJ4 genes (Kurachi, 1995). These channels are largely expressed in atrial, SA and AV nodal cells (Galindo et al, 2016; Navarro-Polanco et al, 2013). At the same time, previous studies (Terzic et al, 1994; Ito et al., 1994) claimed that acetylcholine activates the IK-ATP channels, even though the physiological consequences of this effect on the action potential were not clarified.

The IK-ATP ATP-sensitive potassium channels comprise hetero-octamers consisting of four inward rectifying potassium channel pore-forming subunits (Kir6.1 or Kir6.2, encoded by KCNJ8 and KCNJ11

genes, respectively) and four ATP-binding cassette protein sulphonylurea receptors (SUR1 or SUR2, encoded by ABCC8 and ABCC9 genes, respectively; Inagaki et al, 195). An important feature of the IK-ATP is its closed state under physiological intracellular ATP levels (i. e., under normoxia) and its activation by metabolic stress, when the ratio of ATP/ADP is decreased, e. g., during myocardial ischemia (Deutsch et al., 1991).

Activation of the sarcolemmal IK-ATP during myocardial ischemia shortens the action potential of various cardiac tissues to different extents, thus it may promote APD dispersion and re-entry type arrhythmias (Janse and Wit, 1989). Accordingly, several investigations found IK-ATP activation to be pro-arrhythmic (Chi et al., 1990), suggesting that sarcolemmal IK-ATP inhibition may prevent arrhythmias induced by myocardial ischemia and ischemia/reperfusion (Billman et al, 1998; Englert et al, 2003; Vajda et al, 2007).

In our experiments under normal conditions, we found no effect of carbachol on the membrane current (Figure 3) and, similarly, acetylcholine failed to influence the ventricular and Purkinje APDs (Figures 1A and 1C). The observed discrepancy between our and previous results, where an activation of IK-ATP was described upon acetylcholine administration (Terzic et al, 1994; Ito et al, 1994; Kim et al., 1997), could be the consequence of the species difference and the distinct experimental conditions.

In contrast, an important, and, to the best of our knowledge, previously not published result of our study is that carbachol is able to suppress the pinacidil-activated IK ATP. As a consequence, in parallel tissue action potential experiments, acetylcholine lengthened the APD as long as it was previously shortened by the application of IK ATP-activator pinacidil. Since IK ATP activation could be arrhythmogenic (Chi et al., 1990) by causing an increase in the APD dispersion, this effect of acetylcholine raises the possibility of a novel antiarrhythmic mechanism of the previously described antiarrhythmic effect of parasympathetic activation during hypoxia (Song et al., 1992; Zuanetti et al., 1987; Collins and Billman, 1989).

Our experiments conducted under hypoxic conditions provided similar results (i. e., acetylcholine lengthened the hypoxia-induced shortened ventricular action potential; Figure 4). Even though tissue hypoxia is a complex phenomenon (Carmeliet, 1999), during which several factors change simultaneously (e. g., Ca2+i, Na+i, pH, conductance of gap junctions, membrane potential etc.), it is feasible that IK ATP activation, as a response to ATP depletion, is an important factor in the observed action potential shortening. Since acetylcholine lengthened the action potential under hypoxic conditions, we suggest IK ATP inhibition as a possible underlying mechanism.

Acetylcholine decreased the pinacidil-induced ventricle–Purkinje APD dispersion

Free-running Purkinje fibers connect to the ventricular muscle on a small surface area, providing a relatively large-resistance coupling (Tranum-Jensen et al., 1991), and a large sink for current flow that favors conduction blocks more than other parts of the healthy myocardium. Also, due to the weaker electrotonic coupling, the dispersion of repolarization here can be greater than in other areas (Martinez et al., 2018), causing the Purkinje–ventricle APD ratio to have critical importance in arrhythmia generation. In our experiments, we found significantly greater shortening in Purkinje fibers caused by pinacidil that could be the consequence of the generally weaker repolarization reserve that makes the Purkinje action potential to be more susceptible to any pharmacological interventions (Varró et al, 2000; Baláti et al, 1998). Similarly, acetylcholine exerted larger lengthening in the Purkinje fiber probably by

the same reason that ultimately led to reduced ventricle–Purkinje APD dispersion. The reduction of the ventricle–Purkinje fiber APD dispersion could suppress the arrhythmogenic substrate providing a narrower vulnerable period for a critically timed extrasystole to trigger a life-threatening arrhythmia under hypoxic conditions.

Proposed mechanism

Since inhibition of the K-ATP channels is possible by blocking various PKA-mediated pathways (Tinker et al, 2018.), we suggest that the decrease of cAMP levels caused by the activation of cardiac muscarinic receptors using acetylcholine/carbachol was the factor that decreased the density of the IK-ATP current in patch clamp measurements, leading to the subsequent prolongation observed in action potential durations.

Conclusions

We found that muscarinic agonists inhibit the IK-ATP. Therefore, during IK ATP-mediated action potential shortening, acetylcholine causes asymmetrical action potential lengthening between ventricular muscle and Purkinje fiber that leads to reduced APD dispersion.

These results suggest that the parasympathetic tone beyond suppressing the catecholaminerg-induced arrhythmogenic triggers (Song et al., 1992) may be also able to reduce the arrhythmogenic substrate under hypoxic conditions.

Study Limitations

(i) In our experiments, the ventricular and Purkinje fiber action potentials were measured from electrically uncoupled tissue samples.

(ii) The presented effects were attributed to the M2 muscarinic receptor; nevertheless, the exact level of contribution of other receptor subtypes was not addressed. To achieve this, further studies are needed, utilizing specific agonist and antagonist drugs.

Figures

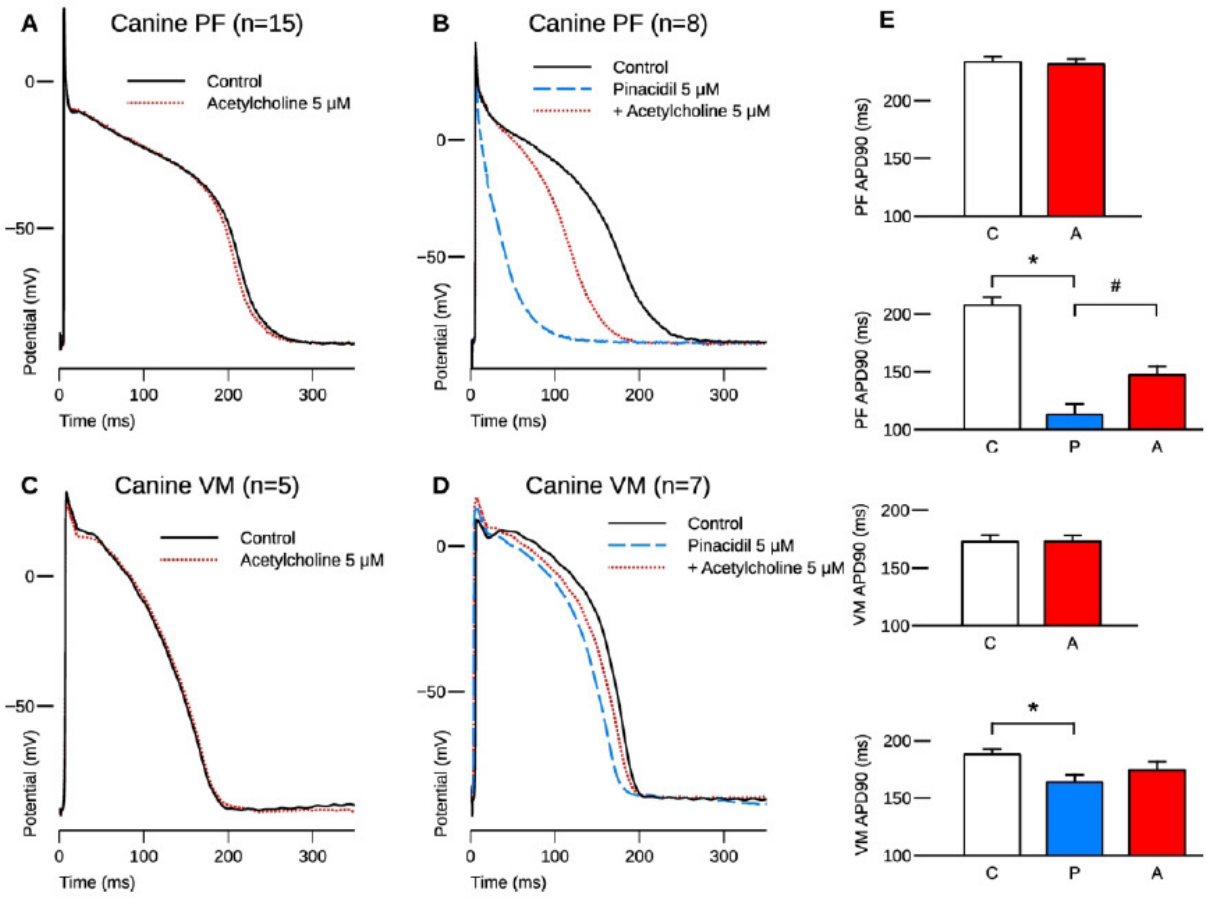


Figure 1. Representative traces of Purkinje fiber (A, B) and ventricular muscle preparations (C, D); 5 µM acetylcholine (red dotted lines) alone caused no changes in either preparation type (A, C), while it caused significant prolongation when applied cummulatively after 5 µMpinacidil (B, D, pinacidil effect represented as blue dashed lines). Bars in panel E represent the values of APD90 in each treatment group, from top to bottom corresponding to the traces A to D. Abbreviations under bars: C, control; P, pinacidil, A, acetylcholine. The pacing cycle length was 500 ms. Values are mean ± SEM; *,# $p<0.05$ RM ANOVA followed by Bonferroni's post-hoc test.

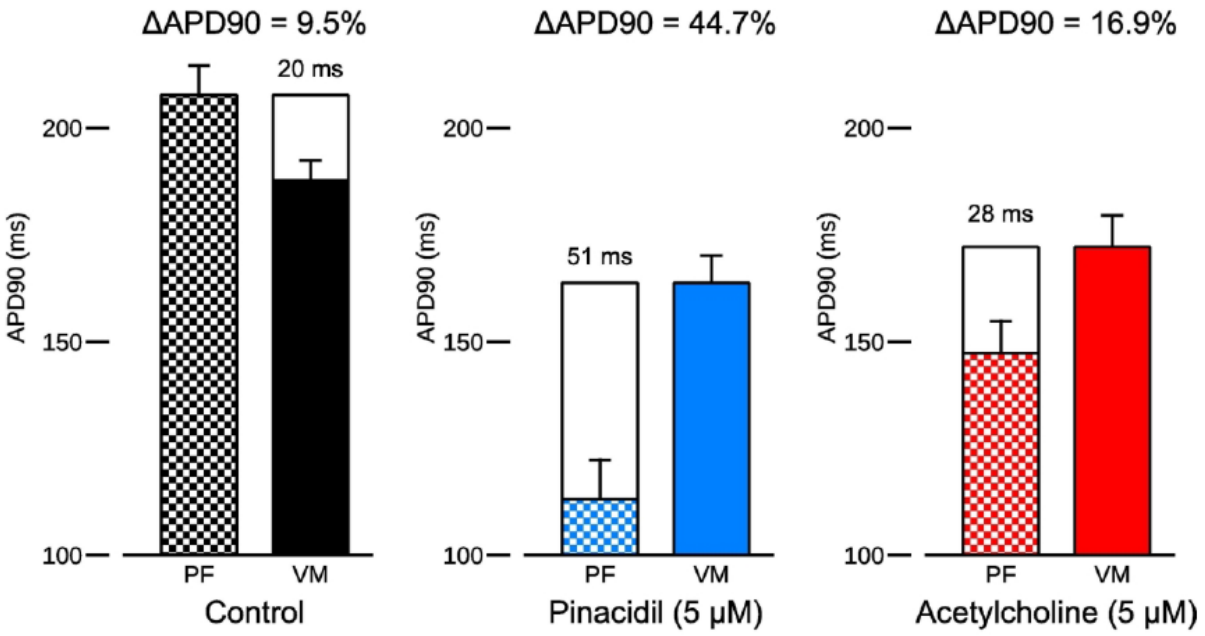


Figure 2. Pinacidil (5 μM) increased the action potential duration dispersion (indicated by ΔAPD90 in percentages, and in ms above the bars) between Purkinje fiber and ventricular muscle preparations, while acetylcholine (5 μM), when applied after pinacidil, decreased dispersion. The pacing cycle length was 500 ms.

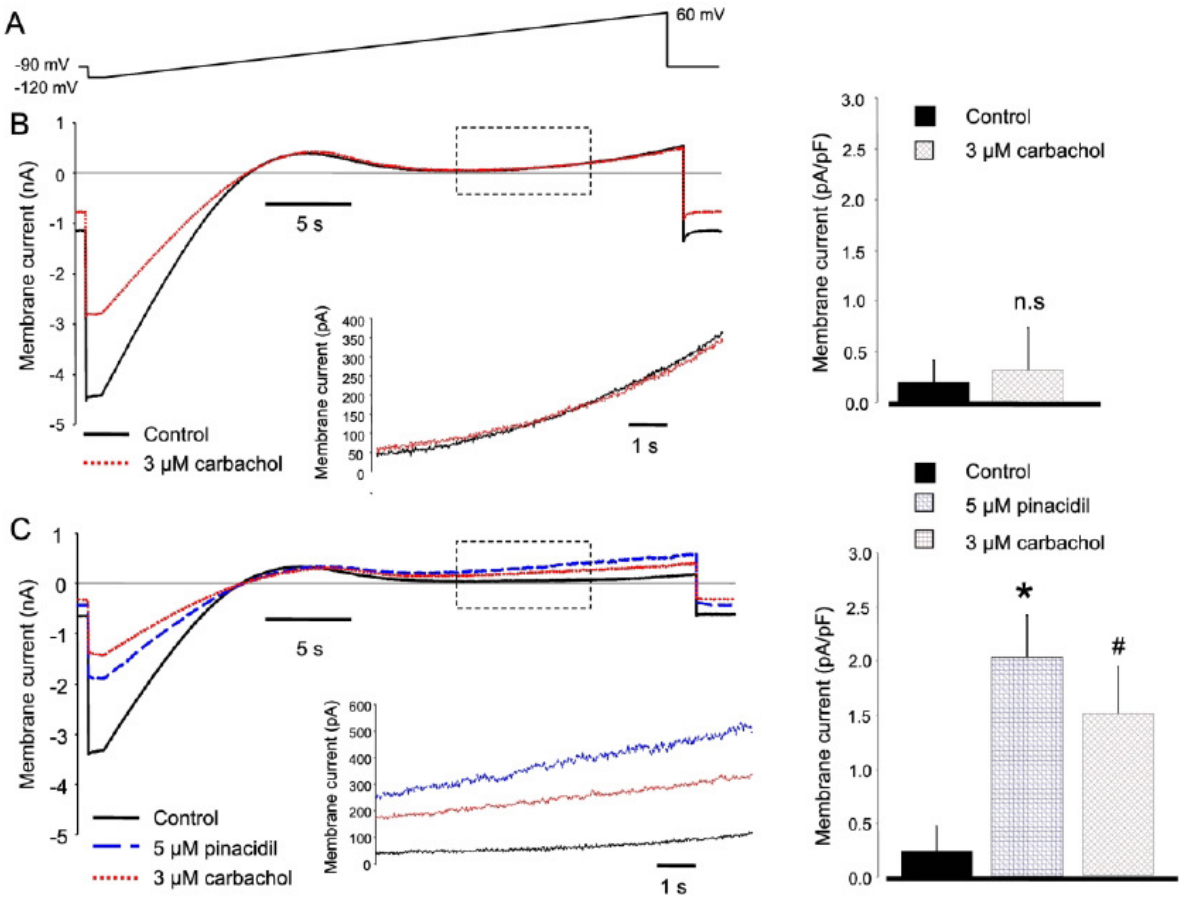


Figure 3. Effect of carbachol on IK-ATP. Ionic currents were measured under a slow voltage ramp protocol (panel A) between -120 mV and 60 mV. The currents were analysed at 0 and 30 mV. Panel B demonstrates original representative current traces (left) and bar graphs (right) where 3 μMcarbachol (dotted line) failed to influence the control current analysed at 0 mV. Inset shows identical current fractions between –3 mV and 45 mV (indicated by dashed rectangle). Current traces in panel C as well as in the inset, illustrate large increase of the membrane current after application of 5 μMpinacidil (blue dashed line) that was inhibited by the subsequently applied 3 μMcarbachol (red dotted line). In bar graphs (right), asterisk denotes significant change between control (left column) and pinacidil (middle column), while hash tag indicates significant change between pinacidil (middle column) and carbachol (right column).

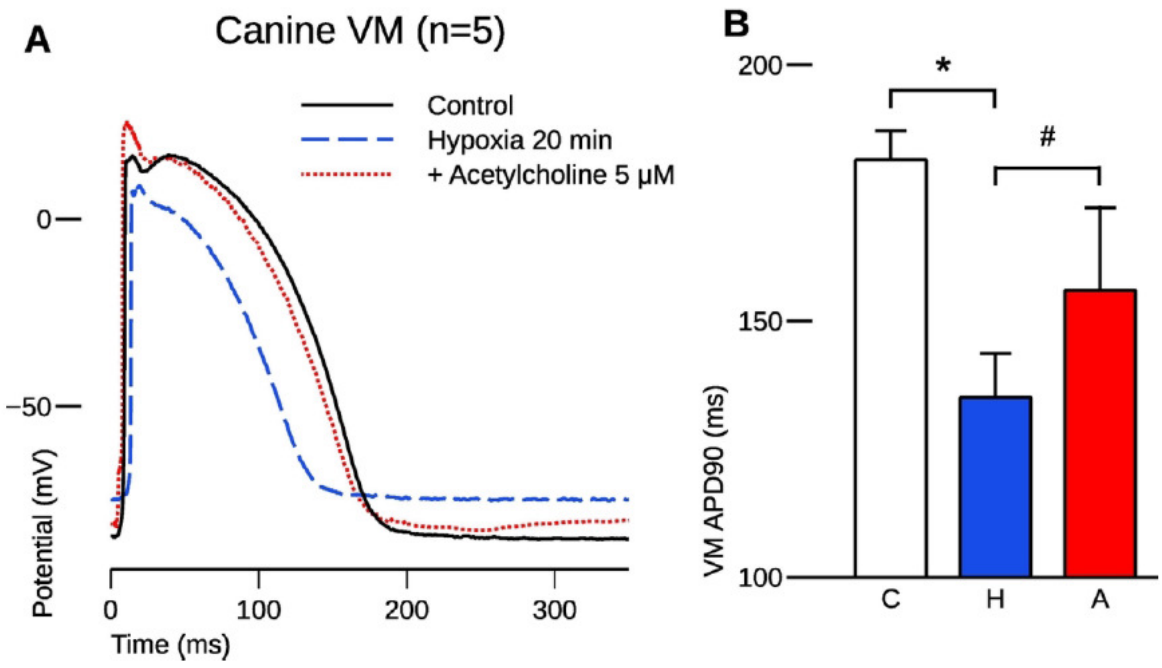


Figure 4. Representative action potential trace (A) showing that hypoxic conditions caused significant action potential duration abbreviation and decreased mean diastolic potential and amplitude in canine ventricular preparations (blue dashed line), while acetylcholine (5 µM) caused a significant prolongation in action potential duration (red dotted line). Values of APD90 are represented as bars (B). Abbreviations under bars: C, control; H, hypoxia, A, acetylcholine. The pacing cycle length was 500 ms. Values are mean ± SEM; *,#p<0.05, RM ANOVA followed by Bonferroni's post-hoc test.

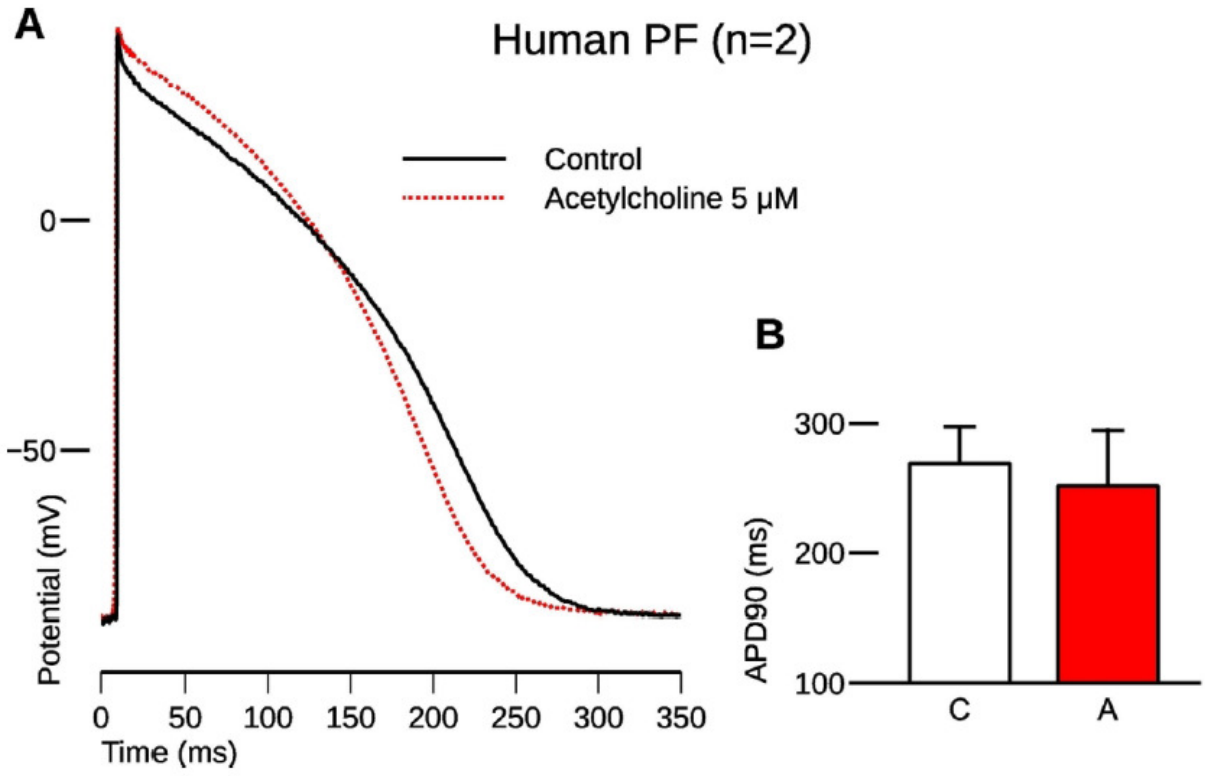


Figure 5. Representative action potential showing the effect of acetylcholine (5 µM, red dotted line) on a Purkinje fiber taken from a human donor heart (A). Values of APD90 are represented as bars (B). Abbreviations under bars: C, control; A, acetylcholine. The pacing cycle length was 500 ms. Values are mean ± SEM.

The APPENDIX of the accepted article contains therapeutic possibility for Early Repolarization Syndrome

Introduction

Acetylcholine has been previously shown to augment J-point elevation and to induce phase-2 reentry, thus precipitating polymorphic ventricular tachycardia in preparations pretreated with agents designed to pharmacologically mimic the genetic defects previously shown to be associated with the early repolarization syndrome (ERS). Previously, Haïssaguerre et al. (2008) have described that extrasystolic activity arising from the Purkinje network is able to precipitate ventricular tachyarrhythmias in the setting of ERS. We examined Purkinje fibers under conditions pharmacologically mimicking the ion channel changes caused by the genetic defects

previously reported to be associated with ERS, including gain of function in $I_{K\text{-}ATP}$ (*KCNJ8* and *ABCC9*) or I_{to} (*SCN1Bb and KCND3*) (Hu et al., 2014b; Barajas-Martínez et al., 2014; Haïssaguerre et al., 2009) or loss of function in I_{Ca} (*CACNA1C, CACNB2* and *CACNA2D1*) (Burashnikov et al., 2010; Napolitano and Antzelevitch, 2011) or I_{Na} (*SCN5A* and *SCN10A*) (Watanabe et al., 2011; Hu et al., 2014a), and applied an antiarrhytmic drug successfully used to treat ventricular tachyarrhythmias in ERS: cilostazol (Iguchi et al., 2013; Shinohara et al., 2014;).

Pharmacological models

Our pharmacological models of the early repolarization syndrome in Purkinje fibers were based on previous experiments (Koncz et al., 2014; Gurabi et al., 2014). We pharmacologically mimicked the ion channel changes caused by the genetic defects associated with ERS: pinacidil (5 μM; $I_{K\text{-}ATP}$ gain of function), NS5806 (7 μM; I_{to} gain of function), nisoldipine (1 μM; I_{Ca} loss of function), mexiletine (20 μM; I_{Na} loss of function). The more efficacious enantiomer of mexiletine, R-mexiletine was used (Gurabi et al., 2017); the concentration corresponds to a peak therapeutic plasma concentration (Varró and Lathrop, 1990). The application of each compound was followed by an equilibration period, enabling the tissue to reach steady-state, then the next compound was administered in a cumulative manner. Acetylcholine (5 μM) was used to simulate increased parasympathetic tone. Cilostazol (10 μM) was applied after acetylcholine.

Results

Model 1: Pinacidil + acetylcholine + cilostazol (n=6)

The effects of pinacidil and acetylcholine were described in the main article. Cilostazol caused a notable plateau elevation without changing repolarization (Figure A1-A).

Model 2: NS5806 + pinacidil + acetylcholine + cilostazol (n=5)

Cilostazol significantly increased action potential duration (APD) when applied after NS5806, pinacidil and acetylcholine (Figure A1-B).

Model 3: Mexiletine + NS5806 + cilostazol (n=4)

After inhibition of I_{Na} by mexiletine, followed by the activation I_{to} by NS5806 and the administration of acetylcholine, cilostazol caused a slight prolongation of the APD (Figure A1-C).

Model 4: Nisoldipine + NS5806 + acetylcholine + cilostazol (n=4)

Cilostazol was also applied after nisoldipine, NS5806 and acetylcholine, causing a slight plateau elevation and slight APD prolongation (Figure A1-D).

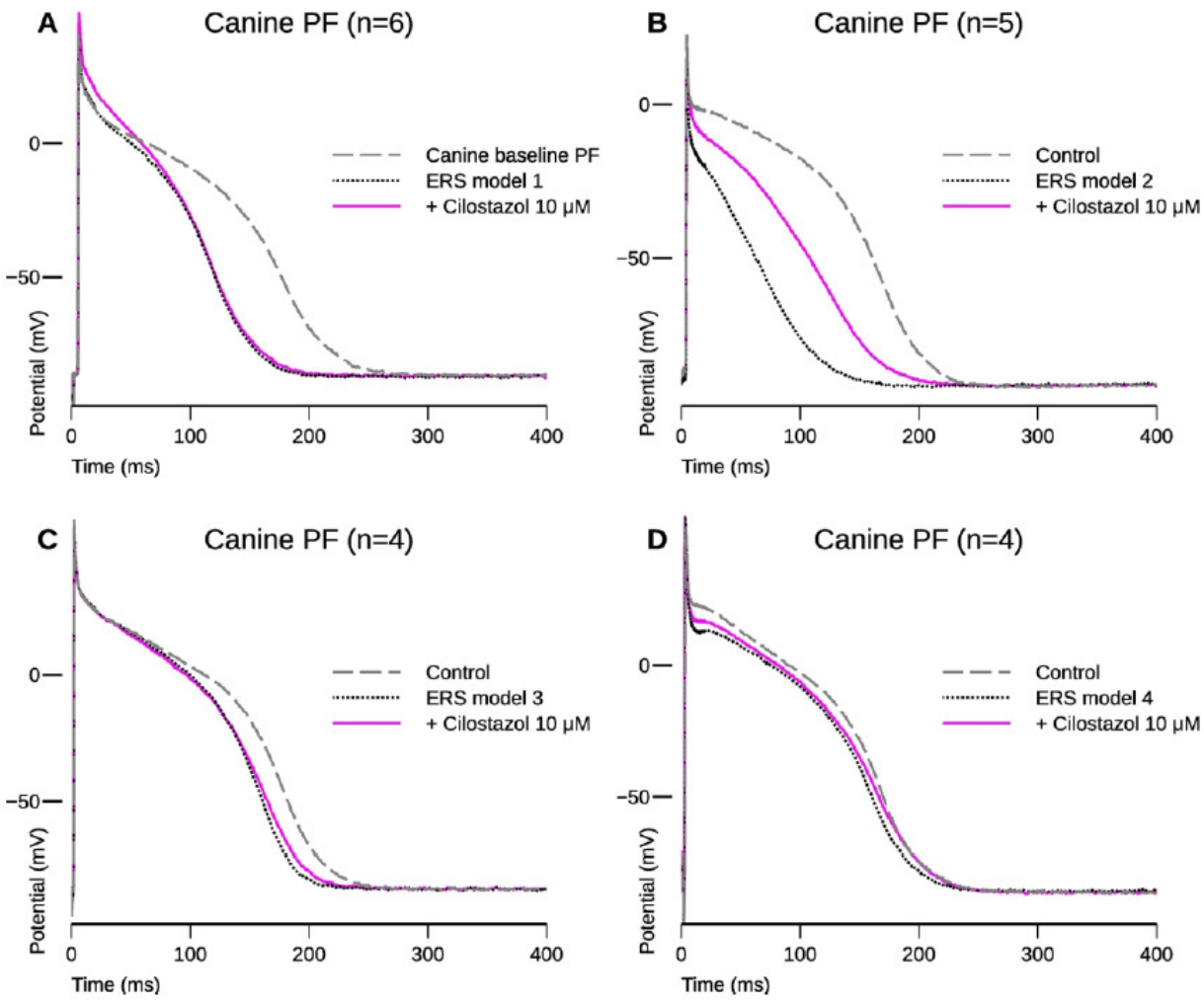


Figure A1. Representative action potential traces from canine Purkinje fibers showing the effects of 10 μM cilostazol (continuous lines) in the following models of the early repolarization syndrome (ERS, dotted lines): pinacidil 5 μM + acetylcholine 5 μM (Model 1; A), NS5806 7 μM + pinacidil 5 μM + acetylcholine 5 μM (Model 2; B), mexiletine 20 μM + NS5806 7 μM (Model 3; C), and nisoldipine 1 μM + NS5806 7 μM + acetylcholine 5 μM (Model 4; D).

Conclusion

Since most conventional antiarrhythmic drugs, including beta-blockers, verapamil, lidocaine or amiodarone, are not capable of suppressing tachyarrhythmic episodes in the early repolarization syndrome, cilostazol should remain a prominent candidate in clinical trials related to early repolarization. Formerly, we found I_{to} blocking ability of cilostazol (Patocskai et al., 2016) next to its ability to augment I_{Ca} (Matsui et al., 1999). The above detailed normalization of the repolarization defect might carry a possible therapeutic value of cilostazol in early repolarization (ER), when the origin of arrhythmic activity is localized to the Purkinje system.

Other results (unpublished)

Comparison (human and dog) of Purkinje fiber action potential parameters (e.g. action potential amplitude)

BCL=500 ms

Baseline electrophysiological parameters of human (preserved ejection fraction, undiseased) ((A) and canine (B) Purkinje fibers.

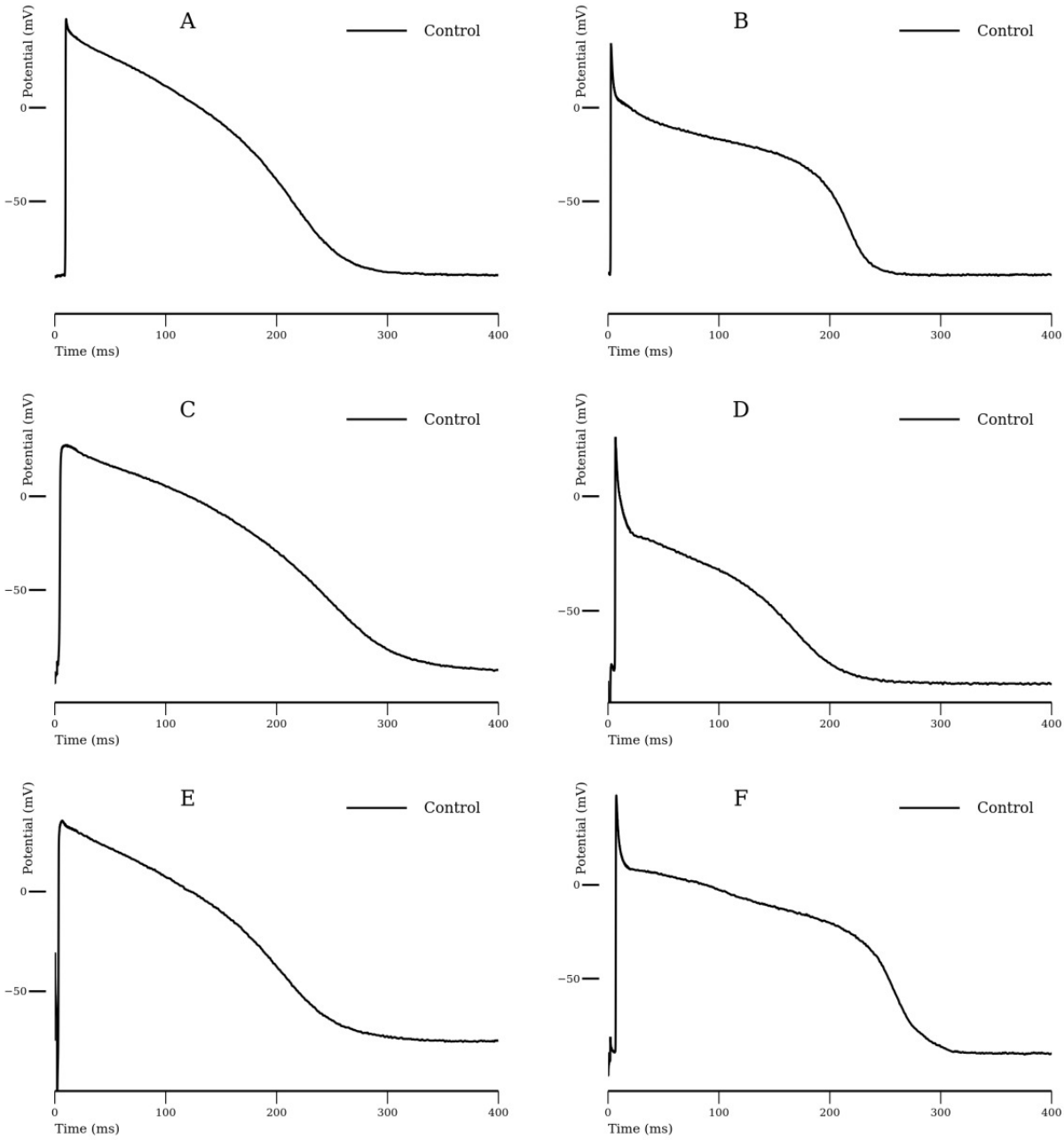
A

PARAMETERS	CT (ms)	RMP (mV)	APA (mV)	APD_{90} (ms)	APD_{75} (ms)	APD_{50} (ms)	APD_{25} (ms)	APD_{10} (ms)	V_{max} (V/s)	PP%
Human Purkinje fibers	5.9±1.9 (3)	−88.5±0.8 (3)	124.2±6.5 (3)	264.9±14.3 (3)	228.7±13.1 (3)	179.2±10.8 (3)	102.7±11.2 (3)	34.1±8.6 (3)	261.2±78.5 (3)	100.2±32.6 (3)

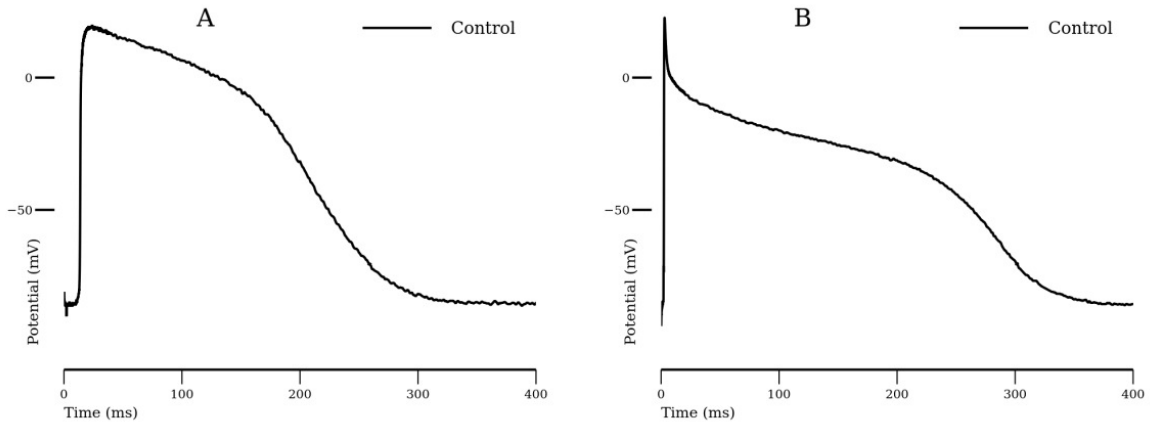
B

PARAMETERS	CT (ms)	RMP (mV)	APA (mV)	APD_{90} (ms)	APD_{75} (ms)	APD_{50} (ms)	APD_{25} (ms)	APD_{10} (ms)	V_{max} (V/s)	PP%
Canine Purkinje fibers	5.3±1.4 (3)	−87.7±1.2 (3)	121.3±9.8 (3)	224.2±25.8 (3)	202.8±29.8 (3)	139.1±40.0 (3)	5.6±0.9 (3)	1.3±0.1 (3)	449.2±32.3 (3)	51.2±3.4 (3)

Means ± SEM.; CT, conduction time; RMP, resting membrane potential; APA, action potential amplitude; APD_{90}, action potential duration at 90% of repolarization; APD_{75}, action potential duration at 75% of repolarization; APD_{50}, action potential duration at 50% of repolarization; APD_{25}, action potential duration at 25% of repolarization; APD_{10}, action potential duration at 10% of repolarization; V_{max}, maximum rising velocity of the action potential upstroke; PP%, plateau potential (expressed as percentage of amplitude of the same action potential); (n), number of observations (i.e., number of preparations taken from different human hearts /A/; number of preparations taken from different canine hearts /B/).

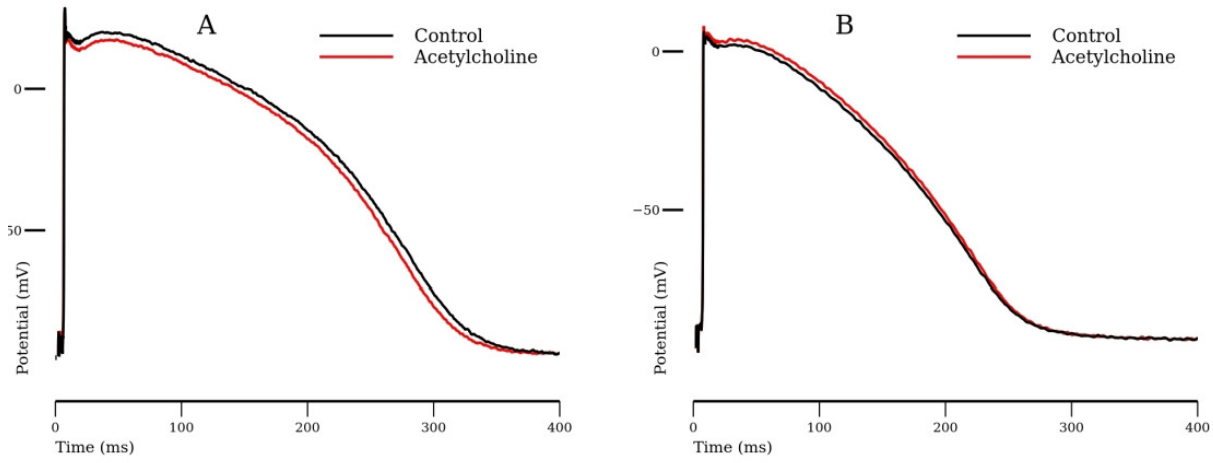


Other results **Figure 1.** Action potential characteristics of baseline (preserved ejection fraction, undiseased) human (A,C,E) and canine (B, D, F) Purkinje fibers (BCL 500 ms)



Other results **Figure 2** Action potential characteristics of a baseline (preserved ejection fraction, undiseased) human (A) and a canine (B) Purkinje fiber (BCL 1000 ms)

Other results **Figure 3**

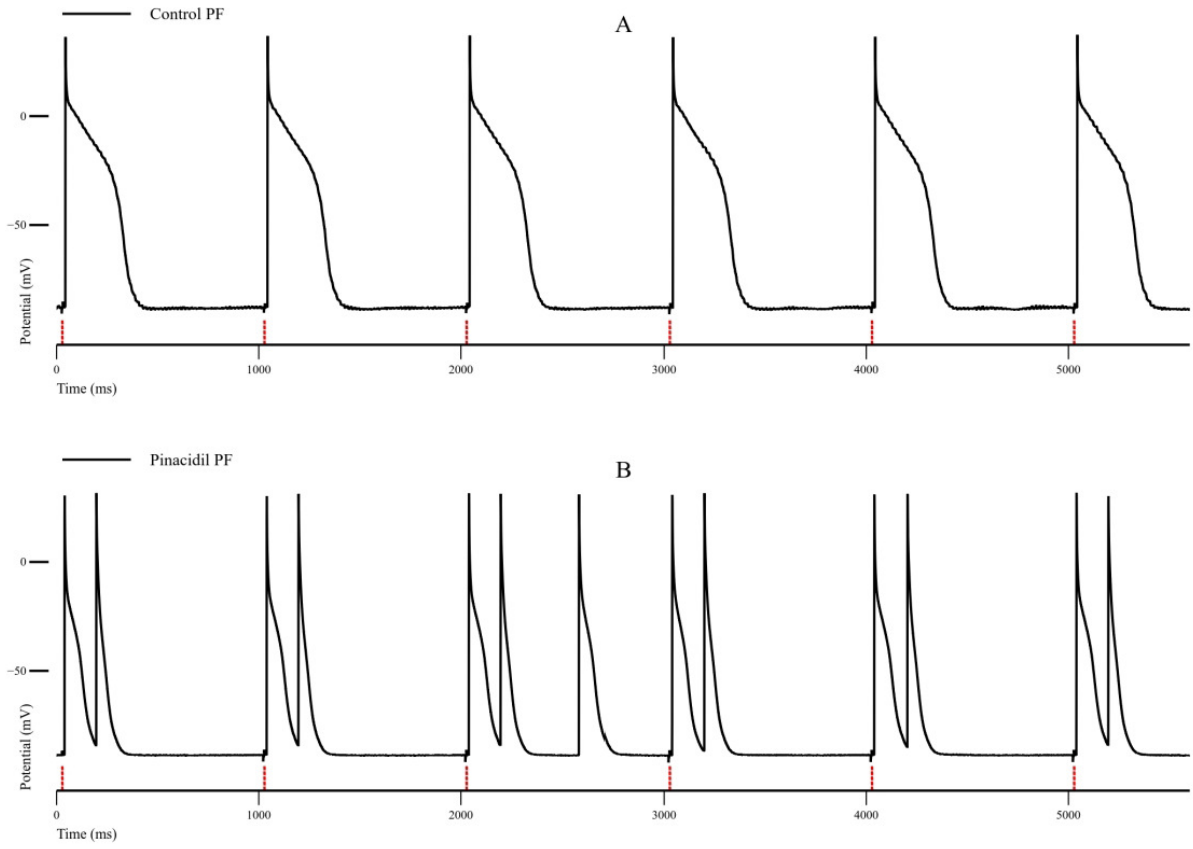


(A) Effects of acetylcholine (5 µM) on action potential waveform of a human subepicardial cell (impaled in a heart slice from undiseased donor heart) at a BCL of 1000 ms after 10 minutes exposure. Note decrease in plateau and reduction in action potential duration.

(B) Effects of acetylcholine (5 µM) on action potential waveform of a human midmyocardial (M) cell (impaled in a heart slice from undiseased donor heart) at a BCL of 500 ms after 3 minutes exposure. Note slight increase in plateau.

Other results

10 µM pinacidil elicited extrasystolic activity in 1/3 dog Purkinje fibres:



Supplementum to final report (NKFIH PD116011 project)

Therapeutic possibilities:

Effect of cilostazol in Early Repolarization Syndrome models (dog Purkinje fibers):

Effects of NS5806, pinacidil, acetylcholine and cilostazol on canine Purkinje fiber action potential characteristics at stimulation cycle length of 1000 ms

PARAMETERS	CT (ms)	RMP (mV)	APA (mV)	APD_{90} (ms)	APD_{75} (ms)	APD_{50} (ms)	APD_{25} (ms)	APD_{10} (ms)	V_{max} (V/s)	PP%
Control	4.3 ± 0.5 (5)	−85.1 ± 1.7 (5)	127.0 ± 5.9 (5)	262.5 ± 20.6 (5)	234.6 ± 20.9 (5)	183.3 ± 19.3 (5)	25.3 ± 7.3 (5)	1.6 ± 0.3 (5)	520.0 ± 116.2 (5)	58.2 ± 1.8 (5)
NS5806 7 µM – 30 min	4.1 ± 0.5 (5)	−85.8 ± 3.2 (5)	124.6 ± 5.1 (5)	263.1 ± 18.3 (5)	238.2 ± 18.3 (5)	182.2 ± 15.7 (5)	15.1 ± 4.6 (5)	1.3 ± 0.1 (5)	534.7 ± 97.5 (5)	56.5 ± 1.4 (5)
Pinacidil 5 µM – 30 min	4.3 ± 0.8 (5)	−84.5 ± 2.4 (5)	126.8 ± 6.6 (5)	127.8 ± 11.9** (5)	101.6 ± 11.6** (5)	48.9 ± 9.9** (5)	3.8 ± 0.5 (5)	1.0 ± 0.1* (5)	553.7 ± 102.2 (5)	41.8 ± 2.9* (5)
Acetylcholine 5 µM – 3 min	4.4 ± 0.8 (5)	−86.0 ± 2.4* (5)	127.0 ± 6.7 (5)	149.8 ± 13.4** (5)	125.5 ± 14.6* (5)	80.5 ± 14.5* (5)	7.3 ± 2.7 (5)	1.2 ± 0.2 (5)	556.7 ± 109.4 (5)	49.8 ± 3.2* (5)
Acetylcholine 5 µM – 30 min	4.6 ± 0.8 (5)	−84.9 ± 2.0 (5)	128.1 ± 6.7 (5)	151.6 ± 9.9* (5)	127.8 ± 10.5* (5)	79.5 ± 11.1* (5)	6.4 ± 2.0 (5)	1.1 ± 0.1* (5)	581.6 ± 81.7 (5)	50.0 ± 2.9 (5)
Cilostazol 10 µM – 30 min	4.2 ± 1.0 (5)	−84.9 ± 0.6 (5)	127.9 ± 7.3 (5)	175.6 ± 7.4* (5)	149.1 ± 6.9 (5)	101.4 ± 6.8 (5)	12.6 ± 3.4 (5)	1.4 ± 0.2 (5)	615.2 ± 96.8 (5)	54.0 ± 1.9 (5)

Means ± SEM.; CT, conduction time; RMP, resting membrane potential; APA, action potential amplitude; APD_{90}, action potential duration at 90% of repolarization; APD_{75}, action potential duration at 75% of repolarization; APD_{50}, action potential duration at 50% of repolarization; APD_{25}, action potential duration at 25% of repolarization; APD_{10}, action potential duration at 10% of repolarization; V_{max}, maximum rising velocity of the action potential upstroke; PP%, plateau potential (expressed as percentage of amplitude of the same action potential); (n), number of observations (i.e., number of preparations obtained from different animals); *p < 0.05; **p < 0.01.

Effects of R-(-)mexiletine, NS5806, acetylcholine and cilostazol on canine Purkinje fiber action potential characteristics at stimulation cycle length of 500 ms (A).

Effects of nisoldipine, NS5806, acetylcholine and cilostazol on canine Purkinje fiber action potential characteristics at stimulation cycle length of 500 ms (B).

A

PARAMETERS	CT (ms)	RMP (mV)	APA (mV)	APD_{90} (ms)	APD_{75} (ms)	APD_{50} (ms)	APD_{25} (ms)	APD_{10} (ms)	V_{max} (V/s)	PP%
Control	3.4 ± 0.5 (4)	−86.5 ± 1.6 (4)	125.3 ± 9.4 (4)	227.6 ± 14.2 (4)	204.6 ± 13.9 (4)	155.0 ± 12.3 (4)	24.7 ± 14.3 (4)	1.8 ± 0.5 (4)	498.0 ± 97.2 (4)	59.0 ± 2.9 (4)
R-(-) mexiletine 20 µM – 30 min	3.8 ± 0.7 (4)	−86.1 ± 0.9 (4)	124.8 ± 8.6 (4)	187.7 ± 6.9 (4)	163.8 ± 6.3 (4)	111.5 ± 8.9 (4)	17.5 ± 5.7 (4)	1.6 ± 0.2 (4)	463.3 ± 62.4 (4)	55.6 ± 3.6 (4)
NS5806 7 µM – 30 min	3.6 ± 0.7 (4)	−88.1 ± 2.0 (4)	128.0 ± 6.4 (4)	190.6 ± 7.9 (4)	165.5 ± 7.0 (4)	112.0 ± 8.6 (4)	13.5 ± 3.6 (4)	1.6 ± 0.1 (4)	477.9 ± 84.4 (4)	53.1 ± 3.3 (4)
Acetylcholine 5 µM – 1 min	3.6 ± 0.7 (4)	−88.7 ± 1.6 (4)	127.7 ± 6.3 (4)	187.9 ± 6.1 (4)	163.5 ± 5.7 (4)	114.7 ± 8.4* (4)	15.3 ± 4.6 (4)	1.7 ± 0.2 (4)	454.1 ± 68.0 (4)	54.2 ± 3.1 (4)
Acetylcholine 5 µM – 30 min	3.9 ± 0.6 (4)	−87.1 ± 2.8 (4)	127.6 ± 6.5 (4)	186.0 ± 5.2 (4)	163.2 ± 4.6 (4)	115.1 ± 7.0 (4)	16.0 ± 4.8 (4)	1.6 ± 0.1 (4)	536.5 ± 58.8 (4)	55.3 ± 2.7 (4)
Cilostazol 10 µM – 30 min	3.8 ± 0.7 (4)	−82.7 ± 3.9 (4)	127.3 ± 8.1 (4)	192.1 ± 7.3 (4)	165.5 ± 6.0 (4)	113.5 ± 8.4 (4)	12.5 ± 3.2 (4)	1.5 ± 0.3 (4)	490.7 ± 66.8 (4)	54.0 ± 2.1 (4)

B

PARAMETERS	CT (ms)	RMP (mV)	APA (mV)	APD_{90} (ms)	APD_{75} (ms)	APD_{50} (ms)	APD_{25} (ms)	APD_{10} (ms)	V_{max} (V/s)	PP%
Control	3.5 ± 0.4 (4)	−86.4 ± 2.5 (4)	132.1 ± 4.5 (4)	211.5 ± 7.5 (4)	187.9 ± 8.7 (4)	133.3 ± 15.1 (4)	21.6 ± 7.2 (4)	1.7 ± 0.4 (4)	494.4 ± 52.0 (4)	56.7 ± 3.7 (4)
Nisoldipine 1 µM – 30 min	3.6 ± 0.5 (4)	−85.3 ± 0.9 (4)	135.7 ± 4.7 (4)	208.4 ± 7.7 (4)	184.3 ± 9.4 (4)	139.0 ± 11.5 (4)	13.7 ± 6.3 (4)	1.7 ± 0.3 (4)	564.0 ± 72.5 (4)	58.2 ± 2.8 (4)
NS5806 7 µM – 30 min	3.6 ± 0.4 (4)	−84.2 ± 1.1 (4)	136.1 ± 5.1 (4)	208.1 ± 7.9 (4)	187.3 ± 9.3* (4)	143.8 ± 11.9 (4)	13.2 ± 6.2 (4)	1.2 ± 0.5 (4)	593.3 ± 37.5 (4)	59.7 ± 2.5 (4)
Acetylcholine 5 µM – 3 min	3.5 ± 0.4 (4)	−84.1 ± 1.0 (4)	131.1 ± 6.8 (4)	206.9 ± 8.5 (4)	187.3 ± 9.2 (4)	147.3 ± 9.3 (4)	14.5 ± 7.8 (4)	1.6 ± 0.3 (4)	611.6 ± 15.1 (4)	60.7 ± 1.5 (4)
Acetylcholine 5 µM – 30 min	3.0 ± 0.4 (4)	−82.7 ± 2.6 (4)	136.6 ± 4.3 (4)	207.4 ± 9.5 (4)	187.6 ± 10.8 (4)	144.3 ± 10.2 (4)	9.4 ± 3.9 (4)	1.4 ± 0.1 (4)	633.6 ± 33.6 (4)	58.7 ± 1.5 (4)

Cilostazol 10 µM – 30 min	3.4 ± 0.4 (4)	−83.7 ± 0.7 (4)	135.6 ± 7.3 (4)	212.3 ± 8.9 (4)	188.2 ± 9.6 (4)	141.7 ± 9.4 (4)	12.7 ± 3.8 (4)	1.6 ± 0.2 (4)	580.5 ± 81.9 (4)	58.6 ± 1.7 (4)

Means ± SEM.; CT, conduction time; RMP, resting membrane potential; APA, action potential amplitude; APD_{90}, action potential duration at 90% of repolarization; APD_{75}, action potential duration at 75% of repolarization; APD_{50}, action potential duration at 50% of repolarization; APD_{25}, action potential duration at 25% of repolarization; APD_{10}, action potential duration at 10% of repolarization; V_{max}, maximum rising velocity of the action potential upstroke; PP%, plateau potential (expressed as percentage of amplitude of the same action potential); (n), number of observations (i.e., number of preparations obtained from different animals); *$p < 0.05$.

Normalization of the repolarization defect might carry a possible therapeutic value of cilostazol in early repolarization syndrome (ERS), when the origin of arrhythmic activity is localized to the Purkinje system.

Istvan Koncz

2020. november 19.