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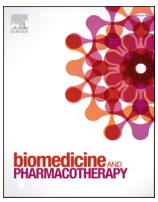

Aminophylline at clinically relevant concentrations affects inward rectifier potassium current in healthy porcine and failing human cardiomyocytes in a similar manner

Markéta Bébarová [a,b,*], Olga Švecová [a], Roman Kula [a], Michal Pásek [a,c], Edita Jeklová [d], Petr Fila [e,f], Martin Pešl [g,h]

[a] Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, Brno 625 00, Czech Republic
[b] Department of Internal Medicine and Cardiology, University Hospital Brno and Faculty of Medicine, Masaryk University, Jihlavská 20, Brno 625 00, Czech Republic
[c] Institute of Thermomechanics, Czech Academy of Sciences, Dolejškova 5, Prague 182 00, Czech Republic
[d] Veterinary Research Institute, Hudcova 70, Brno 621 00, Czech Republic
[e] Centre of Cardiovascular Surgery and Transplantation, Pekařská 53, Brno 602 00, Czech Republic
[f] Department of Cardiovascular Surgery and Transplantation, Faculty of Medicine, Masaryk University, Kamenice 5, Brno 625 00, Czech Republic
[g] ICRC, St. Anne's University Hospital, Pekařská 53, Brno 602 00, Czech Republic
[h] 1st Department of Internal Medicine, Cardio-Angiology, Faculty of Medicine, Masaryk University, Pekařská 53, Brno 602 00, Czech Republic

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ABSTRACT

Aminophylline, a bronchodilator mainly used to treat severe asthma attacks, may induce arrhythmias. Unfortunately, the underlying mechanism is not well understood. We have recently described a significant, on average inhibitory effect of aminophylline on inward rectifier potassium current I_{K1}, known to substantially contribute to arrhythmogenesis, in rat ventricular myocytes at room temperature. This study was aimed to examine whether a similar effect may be observed under clinically relevant conditions. Experiments were performed using the whole cell patch clamp technique at 37°C on enzymatically isolated healthy porcine and failing human ventricular myocytes. The effect of clinically relevant concentrations of aminophylline (10–100 μM) on I_{K1} did not significantly differ in healthy porcine and failing human ventricular myocytes. I_{K1} was reversibly inhibited by ~20 and 30 % in the presence of 30 and 100 μM aminophylline, respectively, at −110 mV; an analogical effect was observed at −50 mV. To separate the impact of I_{K1} changes on AP configuration, potentially interfering ionic currents were blocked (L-type calcium and delayed rectifier potassium currents). A significant prolongation of AP duration was observed in the presence of 100 μM aminophylline in porcine cardiomyocytes which well agreed with the effect of a specific I_{K1} inhibitor Ba^{2+} (10 μM) and with the result of simulations using a porcine ventricular cell model. We conclude that the observed effect of aminophylline on healthy porcine and failing human I_{K1} might be involved in its proarrhythmic action. To fully understand the underlying mechanism, potential aminophylline impact on other ionic currents should be explored.

1. Introduction

Aminophylline, a complex of bronchodilator theophylline and solubility-improving agent ethylenediamine (2:1), is used in clinical practice to treat namely severe asthma attacks [22,24,31,8]. It is also known to be abused by professional athletes who do not suffer from asthma [23]. The administration of aminophylline is associated with an increased risk of tachyarrhythmias, most often atrial fibrillation, even at therapeutic concentrations (*e.g.* [34,6]). Life-threatening ventricular arrhythmias have been described as well (*e.g.* [28,26,14]).

Mechanisms underlying the proarrhythmic aminophylline action are not well understood. As known, aminophylline is a non-specific phosphodiesterase (PDE) inhibitor and an adenosine receptor antagonist [37]. The proarrhythmic action of aminophylline in atria may be related to its positive chronotropic effect, a heterogenous shortening of the atrial effective refractory period (ERP), and a dispersion of recovery of atrial excitability (reviewed by [33]). In ventricles, a significant aminophylline-induced shortening of the ERP was documented in dogs

* Corresponding author at: Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, Brno 625 00, Czech Republic.
E-mail address: mbebar@med.muni.cz (M. Bébarová).

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[20]. Recently, Klimovic et al. [19] have demonstrated an increase in the frequency of rhythm irregularities in both therapeutic and overdose aminophylline concentrations using embryonic bodies formed by human pluripotent stem cell-derived cardiomyocytes. They have suggested a possible role for sarcoplasmic reticulum dysfunction in aminophylline-induced arrhythmogenesis.

Studies focused on the effect of aminophylline/theophylline on the pivotal ionic channels that may affect cardiac repolarization are mostly missing. The only existing study, to our knowledge, is our previous study showing a dual, on average inhibitory, aminophylline effect on inward rectifier potassium (Kir) current (I_{K1}) in rat ventricular myocytes at room temperature [30]. Since changes in Kir currents including I_{K1} may considerably contribute to arrhythmogenesis (*e.g.* [17,13]), this study aimed to explore whether an interaction between aminophylline and I_{K1} might occur in other species and conditions closer to the real clinical situation, namely at 37 °C in healthy porcine and failing human ventricular myocytes.

2. Materials and methods

2.1. Cell isolation

For the isolation of left ventricular cardiomyocytes, we used healthy hearts of 8 pigs (the average weight 50.0 ± 6.1 kg; 3 males and 5 females) and failing hearts of 5 patients (the average age 53.8 ± 6.2 years; for an overview of the basic patients´ data, see Table 1). The porcine hearts were explanted from the cadavers of healthy pigs immediately after their euthanasia in deep anesthesia induced by a combination of tiletamine, zolazepam, ketamine, and xylazine (2 mg/kg of the body weight for all substances; Veterinary Research Institute, Brno, Czech Republic). All experiments using human failing hearts were performed under the ethical standards of the Centre of Cardiovascular Surgery and Transplantation, Brno, Czech Republic, and approved by the Ethics Committee of the Centre. The informed consent of all patients, attachment number 18, from Mar 18, 2020, is archived.

The pig heart was quickly removed, a branch of the left coronary artery was immediately cannulated, and the respective region of the left ventricle was perfused with an ice-cold cardioplegic solution for approx. 5 min (composition in mM: NaCl 110, KCl 16, $NaHCO_3$ 10, $MgCl_2$, 16, $CaCl_2$ 0.6; pH was adjusted to 7.8 with NaOH). Then, the heart was placed into the same ice-cold cardioplegic solution and quickly transported to the laboratory. In the case of the explanted human heart, it was put into the ice-cold cardioplegic solution right in the operating room, transported quickly to the laboratory, and a branch of the left coronary artery was cannulated. Subsequently, the same procedure was applied to the pig and human hearts to obtain the isolated left ventricular myocytes. The cannulated heart was attached to a gravity-driven Langendorff apparatus. The region of interest was sequentially perfused with the following solutions (oxygenated with 100 % O_2 and warmed to 37 °C): (i.) a nominally Ca-free Tyrode solution (0.6 μM Ca^{2+}, ~10 min), (ii.) with the same solution supplemented with collagenase (Collagenase A, Roche Diagnostics GmbH, Mannheim, Germany; 1 mg/ml, 34.9 ± 1.7 min in the pig hearts and 33.4 ± 3.7 min in the human hearts), and (iii.) finally with the nominally Ca-free Tyrode solution again (~10 min). Then the heart was removed from the perfusion apparatus and gelatinous myocardial tissue in the perfused region was dissected and placed into the nominally Ca-free Tyrode solution (37 °C). The tissue was cut and filtered through a nylon mesh. The final cell suspension was left to sediment. After ~10 min, the supernatant was removed and replaced with a fresh nominally Ca-free Tyrode solution. The cells were washed in this way three times. During the final step, the spontaneous sedimentation of the cells was replaced with centrifugation of the suspension (400 rpm, 3 min). Then, the suspension of cells was exposed to an increased Ca^{2+} concentration of 1.8 mM, and the cells were left to adapt for an hour before the patch clamp measurements.

Table 1
Clinical characteristics of patients.

Patient No.	Sex	Age (years)	Main diagnosis	Ejection fraction (%)
Patient 1	male	51	restrictive cardiomyopathy	40
Patient 2	female	65	dilation cardiomyopathy	20
Patient 3	male	32	Becker's dystrophy	21
Patient 4	male	66	dilation cardiomyopathy	25
Patient 5	male	55	ischemic heart disease	19

The ejection fraction was determined according to the Teichholz formula (before the heart transplantation).

2.2. Solutions and chemicals

Tyrode solution of the composition below was used during both the dissociation procedure and perfusion of the cells during I_{K1} and AP measurements (in mM): NaCl 135, KCl 5.4, $MgCl_2$ 0.9, HEPES 10, NaH_2PO_4 0.33, and glucose 10 (pH was adjusted to 7.4 with NaOH). This solution was supplemented with 1.8 mM $CaCl_2$ during the patch clamp recordings. To keep the experimental conditions used in our previous study dealing with the effect of aminophylline on I_{K1} in rat ventricular myocytes [30], $CoCl_2$ (2 mM) and tetraethylammonium chloride (TEA, 50 mM), respectively, were applied to inhibit calcium current I_{Ca} and the delayed rectifier potassium current I_K in the course of the experiments. The patch electrode filling solution contained the following (in mM): L-aspartic acid 120, KCl 15, $MgCl_2$ 1, K_2ATP 5, EGTA 1, HEPES 5, GTP 0.1, Na_2-phosphocreatine 3 (pH 7.25 adjusted with KOH).

I_{K1} was evaluated as the current sensitive to 100 μM Ba^{2+} similarly as it was done in our previous papers (*e.g.* [4,30]). Although it is unlikely to activate ATP-sensitive potassium current $I_{K(ATP)}$ under the given experimental conditions (*i.e.* 5 mM ATP in the pipette solution, isolated cells), the inhibitor of $I_{K(ATP)}$ glibenclamide (10 μM) was present in all the performed experiments. Atropin (1 μM) was also added to avoid contamination of the measured I_{K1} by the acetylcholine-sensitive current $I_{K(ACH)}$.

$CoCl_2$, atropin, and $BaCl_2$ were prepared as 1 M, 1 mM, and 10 mM stock solutions, respectively, in the deionized water and held at 4° C. Glibenclamide was prepared as 100 mM stock solution in dimethyl sulfoxide (DMSO). The final concentration of DMSO was identical in the control and test solutions (0.01 %); this concentration seems to have no considerable effects on the cardiac I_{K1} [25,5]. To prepare the TEA-containing stock solution, NaCl in the used Tyrode solution (described above) was replaced equimolarly by TEACl. The stock solution of aminophylline was prepared as a fresh 100 mM solution before each measurement (dissolved in deionized water). Aminophylline was added to the Tyrode solution to obtain the final concentrations between 10 and 100 μM. The solutions were applied near the measured cell *via* an electronically operated perfusion system.

The chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic) unless otherwise stated.

2.3. Electrophysiological measurements and evaluation

Single rod-shaped cells with well-visible striations were used for the current and voltage recordings applying the whole-cell patch-clamp technique in the voltage and current clamp modes, respectively. The patch pipettes were pulled from borosilicate glass capillary tubes and heat-polished on a programmable horizontal puller (Zeitz-Instrumente Vertriebs GmbH, Martinsried, Germany). The resistance of the filled glass electrodes was below 1.5 MΩ to keep the access resistance as low as possible. For the generation of experimental protocols and data acquisition, the Axopatch 200B equipment and pCLAMP 9.2 software (Molecular Devices, Sunnyvale, California, USA) were used. The series resistance was compensated up to 60 %. The capacitance was not

compensated because the contribution of capacity current to the measured current was regarded as negligible. The measured ionic currents were digitally sampled at 5 kHz and stored on the hard disc. The holding potential was −85 mV and the stimulation frequency was 0.2 Hz. I_{K1} was evaluated as the Ba^{2+}-sensitive current at the end of 500-ms pulses, either to −50 mV (the outward component) or to −110 mV (the inward component); the sodium current I_{Na} was inactivated during the first pulse to −50 mV. Action potentials (APs) were elicited using 0.5-ms suprathreshold current pulses at the stimulation frequency 1 Hz (sampling rate 10 kHz). The data were corrected for the estimated junction potential by shifting all voltage values by −10 mV. All measurements were performed at 37 °C.

2.4. Mathematical simulations

The simulations were performed using the CellML code of a recently published mathematical model of porcine ventricular cardiomyocyte [12] in the computational environment OpenCore v. 0.6. The code is available in the Supplementary Materials of the paper by Gaur et al. [12].

2.5. Statistical analysis

The normality of data distribution was tested using the Shapiro-Wilk test. Data are presented as mean ± S.E.M. from *n* cells/subjects (biological replicates in all cases). Parametric statistical tests (the paired *t*-test and one-way ANOVA with the Bonferroni post-test as individually specified in respective figure legends) were used to test the statistical significance of the observed differences; $P < 0.05$ was considered statistically significant. The software Origin 2022b (version 9.9.5.171; OriginLab Corporation) and GraphPad Prism 9 (version 9.5.1; GraphPad Software, Inc.) were used for the analysis.

3. Results

3.1. Effect of clinically relevant concentrations of aminophylline on I_{K1} in healthy porcine ventricular myocytes

As illustrated in Fig. 1A, I_{K1} was recorded at −50 and −110 mV which enabled the detection of the changes in its outward (repolarizing) and inward (depolarizing) components, respectively. The magnitude of I_{K1} was evaluated as the current sensitive to 100 μM Ba^{2+} at the end of 500-ms pulses (see the arrows in Fig. 1A) to avoid a contribution of any

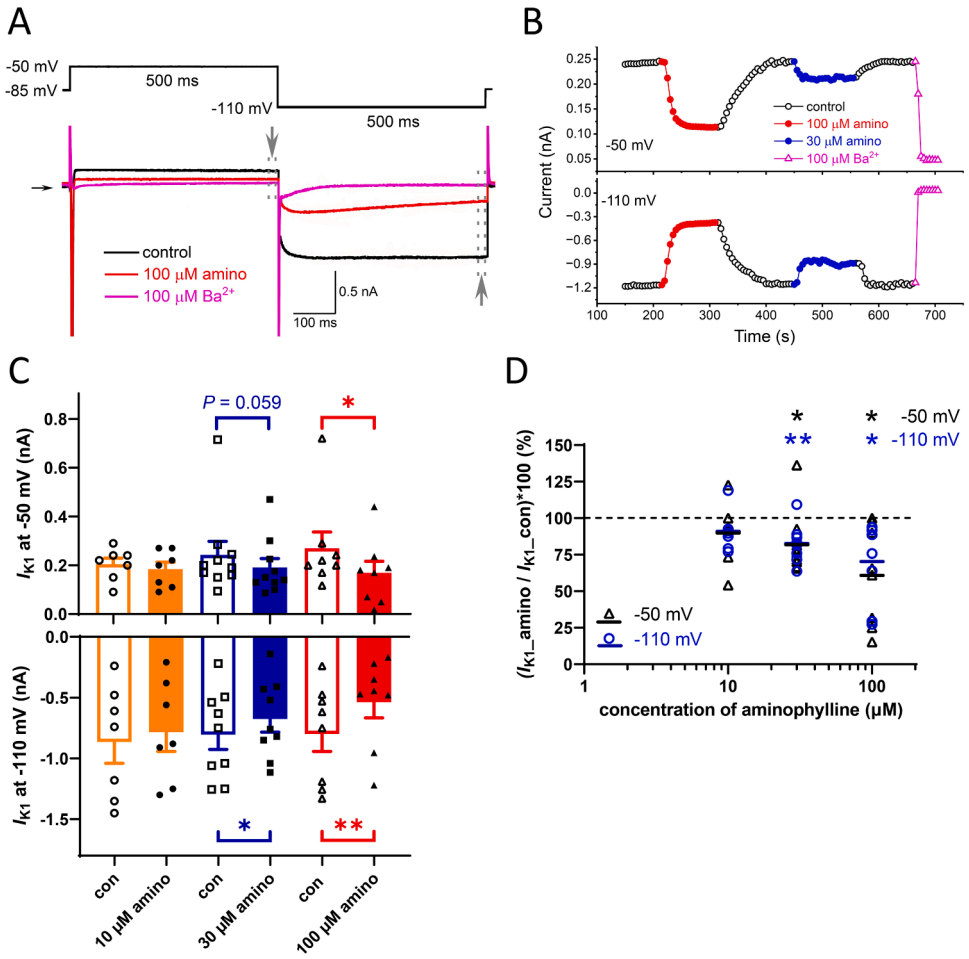

Fig. 1. Changes in inward rectifier potassium current I_{K1} at −50 and −110 mV in healthy porcine left ventricular cardiomyocytes in the presence of 10, 30, and 100 μM aminophylline (amino). A and B: An example of I_{K1} traces (A; I_{K1} was evaluated as the mean current sensitive to 100 μM Ba^{2+} at the end of the depolarizing pulse to −50 mV, *i.e.* the outward component of I_{K1}, and at the end of the repolarizing step to −110 mV, *i.e.* the inward component of I_{K1} – see the grey arrows) and time course of I_{K1} changes during application of 30 and 100 μM aminophylline (B). **C:** Average I_{K1} at −50 mV (upper panel) and −110 mV (lower panel) in 10, 30, and 100 μM aminophylline (amino) and respective controls ($n = 7/4$, 10/6, and 8/5 at −50 mV, and $n = 7/4$, 9/6, and 8/5 at −110 mV). **D:** Concentration dependence of the effect of aminophylline at clinically relevant concentrations between 10 and 100 μM on I_{K1}. To assess the statistical significance of the absolute (B) and relative (C) I_{K1} changes under aminophylline *vs.* the respective control, a paired *t*-test was used; * and * * - statistically significant differences at $P < 0.05$ and 0.01, respectively.

time-dependent currents that were not inhibited pharmacologically, *e.g.* sodium current I_{Na} which was activated and inactivated at the beginning of the pulse to −50 mV. In most examined healthy porcine ventricular myocytes, aminophylline caused an inhibition of I_{K1} which increased with the applied concentration between 10 and 100 μM (for a record during application of 30 and 100 μM aminophylline and 100 μM Ba^{2+}, see Fig. 1B). The average I_{K1} inhibition (Fig. 1C) was significant at 30 and 100 μM aminophylline reaching 17.4 ± 4.2 and 29.7 ± 9.8 %, respectively, at −110 mV (n = 9/6, $P < 0.05$, and n = 8/5, $P < 0.01$, respectively) and 18.2 ± 6.6 and 39.2 ± 11.9 %, respectively, at −50 mV (n = 10/6, $P = 0.059$, and n = 8/5, $P < 0.05$, respectively). At 10 μM aminophylline, the inhibition was insignificant at both tested voltages (9.0 ± 5.2 and 10.4 ± 8.1 % at −110 and −50 mV, respectively; n = 7/4 and $P > 0.05$ at both voltages). In a single cell, we observed an activation of I_{K1} (for an example of I_{K1} activation at 30 μM aminophylline, see Fig. 2C, middle and right lower panels) similarly as we did in our previously published study dealing with the effect of aminophylline on rat ventricular I_{K1} (for the data and detail explanation of the dual aminophylline effect, see [30]). Both activation and inhibition of I_{K1} by aminophylline were fully reversible during the following wash-out (as illustrated in Fig. 1B in the case of the inhibition). The effects did not significantly differ at −50 and −110 mV. The concentration dependence

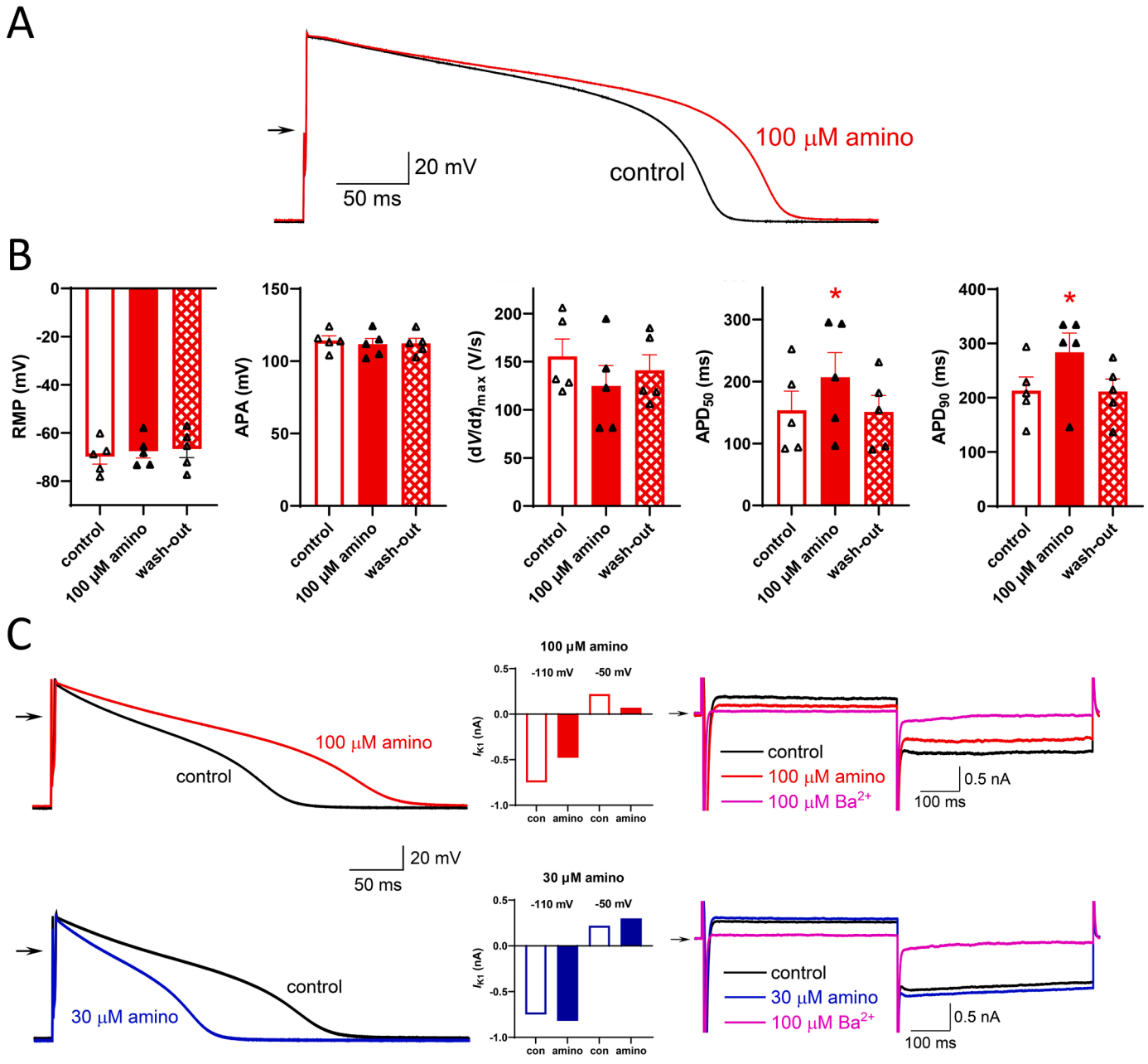

Fig. 2. Effect of 100 μM aminophylline (amino) on action potential (AP) configuration in healthy porcine left ventricular cardiomyocytes at 37°C (recorded in the presence of I_{Ca}, I_{Kr}, and I_{Ks} inhibitors to separate the impact of aminophylline-induced I_{K1} changes). A: An example of AP waveforms in control conditions (black line) and under the effect of 100 μM aminophylline (red line; for another example, please see Fig. 2C, left upper panel). B: Basic AP characteristics (n = 5/3); RMP – resting membrane potential, APA – AP amplitude, APD_{50} – AP duration at 50 %-repolarization, APD_{90} – AP duration at 90 %-repolarization; * - statistically significant differences at $P < 0.05$ (paired t-test *vs.* the respective control). **C:** An example of porcine cardiomyocyte showing a dual aminophylline effect on both AP duration (left panel) and I_{K1} (middle and right panels). Aminophylline at 100 μM induced an inhibition of I_{K1} (middle and right upper panels) and consequent AP prolongation (left upper panel) whereas 30 μM aminophylline resulted in opposite effects, *i.e.* I_{K1} activation (middle and right bottom panels) and AP shortening (left bottom panel).

of the aminophylline effect is shown in Fig. 1D.

3.2. Changes in action potential configuration in the presence of 100 μM aminophylline in healthy porcine ventricular myocytes

Subsequently, we tested if the effect of aminophylline on I_{K1} observed in healthy porcine ventricular myocytes (Fig. 1) may lead to any changes in action potential (AP) configuration in these cells (Fig. 2). To separate the impact of aminophylline-induced I_{K1} changes, we decided to analyze the effect of 100 μM aminophylline on APs under the absence of I_{Ca} and both rapid and slow components of I_K, I_{Kr}, and I_{Ks}, respectively, *i.e.* under the same experimental conditions used during I_{K1} measurements (see Materials and methods). No significant aminophylline-induced changes were apparent in the case of the maximal upstroke velocity ($(dV/dt)_{max}$), action potential amplitude (APA), and resting membrane potential (RMP; for all the parameters, $n = 5$, $P > 0.05$, Figs. 2A and 2B). In contrast, AP duration (APD) was significantly prolonged (Figs. 2A and 2B), both at 50 %- and 90 %-repolarization (APD_{50} and APD_{90}, respectively), APD_{50} from 153.5 ± 31.0 ms in control conditions to 206.9 ± 39.9 ms in the presence of 100 μM aminophylline (*i.e.* prolongation by ~35 %; $n = 5/3$, $P < 0.05$) and APD_{90} from 213.0 ± 25.2 ms in control conditions to 283.8 ± 35.2 ms in the presence of 100 μM aminophylline (*i.e.* prolongation by ~33 %; $n = 5/3$, $P < 0.05$). APD changes were fully reversible during the subsequent wash-out. These findings agreed well with simulations performed in a porcine ventricular cell model (Fig. 5; for details, see Discussion). Moreover, we observed similar changes in AP characteristics under the effect of 10 μM Ba^{2+} causing partial inhibition of I_{K1} (Fig. 3; recorded in the absence of I_{Ca}, I_{Kr}, and I_{Ks} inhibitors). In this case, a significant prolongation of both APD_{50} and APD_{90} by ~14 and 20 %, respectively ($n = 7/2$, $P < 0.05$ and 0.001, respectively) was accompanied by other tiny, but significant changes including an expected depolarizing shift of RMP by 2.03 mV ($n = 7/2$, $P < 0.05$).

As documented in Fig. 2C, we observed a dual impact of aminophylline-I_{K1} interaction in a single cell. When 100 μM aminophylline was applied, I_{K1} was inhibited and AP was prolonged in this cell (Fig. 2C, upper panels) in agreement with the average data from 5 measured cells (Fig. 2B). In contrast, 30 μM aminophylline induced an activation of I_{K1} and consequent AP shortening in the same cell (Fig. 2C, bottom panels). Therefore, heterogeneity in cardiac repolarization might be a potentially proarrhythmic consequence of aminophylline treatment (see Discussion).

3.3. Aminophylline-induced changes in I_{K1} investigated in porcine, human, and rat ventricular myocytes: an interspecies comparison

To approach even more clinically relevant conditions, we further analyzed the effect of 10 – 100 μM aminophylline in human ventricular myocytes freshly isolated from the failing hearts explanted during transplantation under the same experimental conditions. As in the case of I_{K1} in healthy porcine cardiomyocytes (Fig. 1), aminophylline showed an inhibitory action in failing human cardiomyocytes at both −110 and −50 mV (for example, an average inhibition by 23.3 ± 6.5 and 25.7 ± 3.4 % in the presence of 30 μM aminophylline at –110 and −50 mV, respectively; $n = 8/5$ and 6/5, respectively; $P < 0.01$ and $P < 0.05$, respectively, if the absolute values were compared, and $P < 0.01$ and $P < 0.001$, respectively, if the relative values were compared; Fig. 4B and Fig. 4C, upper panel). At 100 μM aminophylline and −50 mV (Fig. 4B, upper panel), the significance was missing if the absolute current values were compared (likely due to high variability of the data), but the effect was significant if the relative values were compared ($P < 0.01$; Fig. 4C, upper panel).

The relative effect observed in failing human cardiomyocytes at 37 °C (Fig. 4C, upper panel) was similar to that examined in healthy porcine ventricular cardiomyocytes at 37 °C in this study (Fig. 4C, middle panel) as well as to the effect investigated in healthy rat ventricular

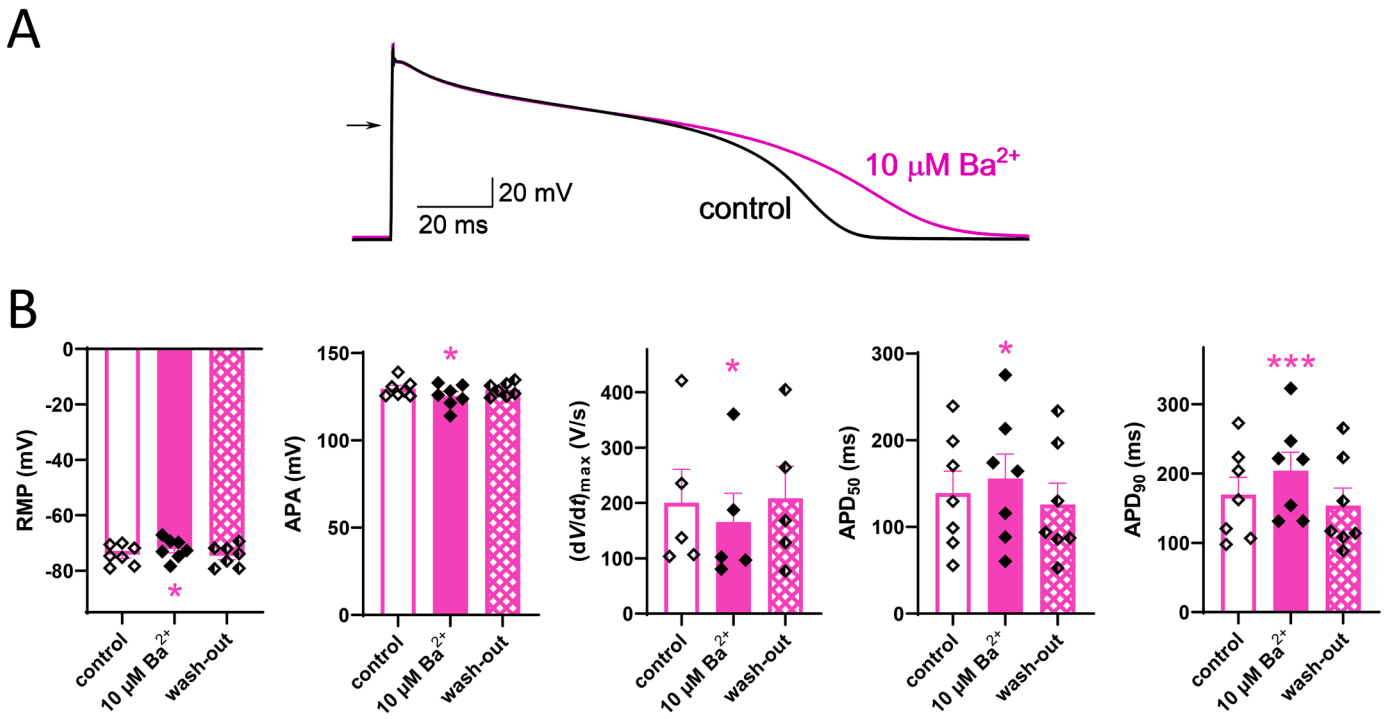

Fig. 3. Effect of a specific I_{K1} inhibitor Ba^{2+} in the concentration of 10 μM causing partial I_{K1} inhibition on action potential (AP) configuration in healthy porcine left ventricular cardiomyocytes at 37°C (without other ionic channel inhibitors). A: Representative AP waveforms in control conditions (the black line) and in the presence of 10 μM Ba^{2+} (the magenta line). B: Basic AP characteristics under the effect of a specific I_{K1} inhibitor 10 μM Ba^{2+} ($n = 7/2$); RMP – resting membrane potential, APA – AP amplitude, APD_{50} – AP duration at 50 %-repolarization, APD_{90} – AP duration at 90 %-repolarization; * and * ** - statistically significant difference at 10 μM Ba^{2+} *vs.* control at $P < 0.05$ and 0.001, respectively (paired *t*-test).

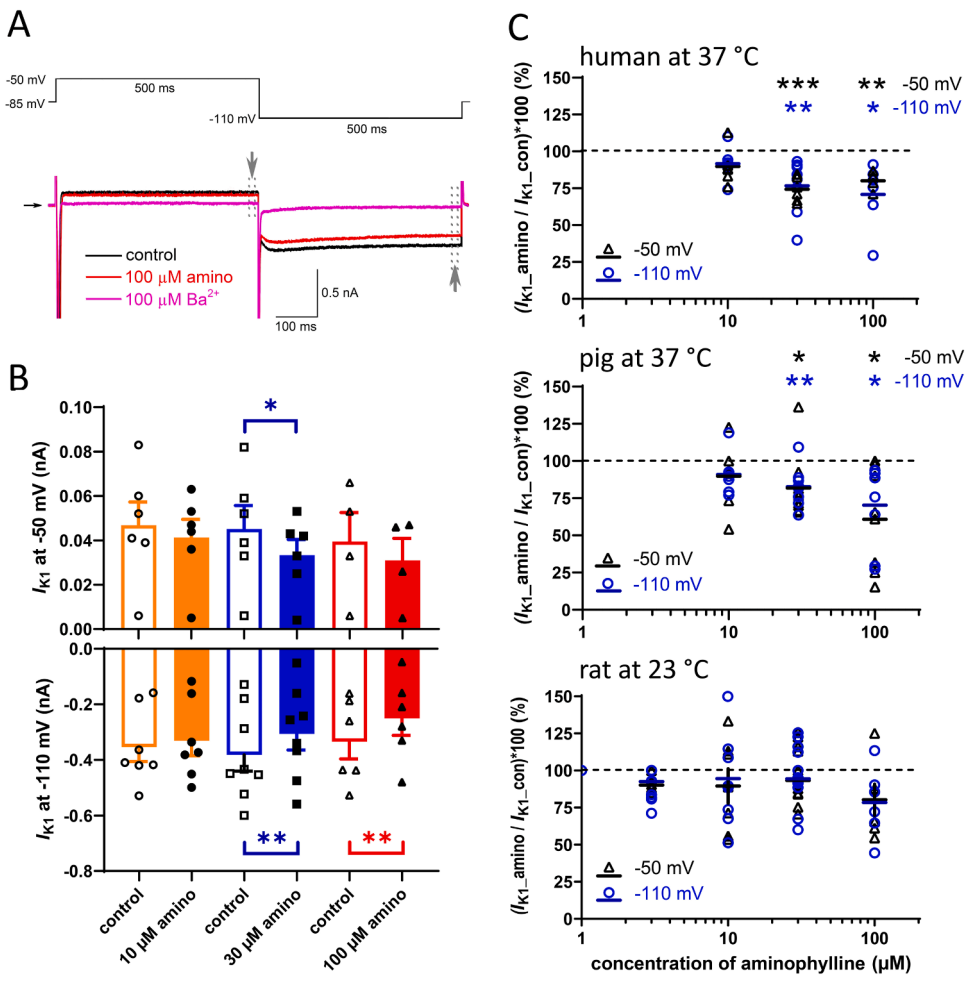

Fig. 4. Effect of aminophylline on I_{K1} in failing human cardiomyocytes. A: Scheme of the experimental protocol and representative I_{K1} traces in control, under 100 μM aminophylline, and specific I_{K1} inhibitor Ba^{2+} in the concentration of 100 μM. B: Average I_{K1} at −50 mV (upper panel) and at −110 mV (lower panel) in 10, 30, and 100 μM aminophylline (amino; dots, squares, and triangles, respectively, full symbols) and respective controls (dots, squares, and triangles, respectively, empty symbols; n = 6/5, 6/5, and 4/3 at −50 mV, and n = 7/5, 8/5, and 6/4 at −110 mV). **C:** Concentration dependence of the effect of 10–100 μM aminophylline on I_{K1} in human failing cardiomyocytes at 37 °C (for the respective n, see the legend A), in healthy porcine ventricular myocytes at 37 °C (n = 7/4, 10/6, and 8/5 at −50 mV, and n = 7/4, 9/6, and 8/5 at −110 mV), and in rat ventricular myocytes at 23 °C for comparison (the rat data were reused from our previously published paper, [30]; n = 10/8, 12/8, and 6/4 at −50 mV, and n = 11/8, 12/8, and 6/4 at −110 mV). To assess the statistical significance of the absolute (B) and relative (C) I_{K1} changes under aminophylline *vs.* the respective control, a paired *t*-test *vs.* the respective control was used; * , * *, and * ** - statistically significant differences at $P < 0.05$, 0.01, and 0.001, respectively. Except for the effect of 30 μM aminophylline at −50 mV in human and rat cardiomyocytes ($P < 0.05$), no statistically significant difference was observed in the relative effect of aminophylline at a given concentration between 10 and 100 μM in the investigated failing human and healthy pig and rat cardiomyocytes (one-way ANOVA with the Bonferroni post-test).

cardiomyocytes at 23 °C (Fig. 4C, lower panel; as published in our recent study [30]); the only significant difference was between the effect of 30 μM aminophylline in human and rat due to the dual effect present in rat, but not in human cardiomyocytes. The average changes were not significantly different at both tested voltages for any of the applied concentrations ($P > 0.05$).

4. Discussion

In this study, we first proved that aminophylline at clinically relevant concentrations of 30 and 100 μM exerted a comparable, on average inhibitory, effect on inward and outward components of I_{K1} in healthy porcine and failing human ventricular myocytes (Figs. 1 and 4). If other pivotal currents playing a role in AP plateau and repolarization, namely I_{Ca}, I_{Kr}, and I_{Ks}, were inhibited to separate the impact of I_{K1} changes on AP configuration, 100 μM aminophylline resulted in AP prolongation (Fig. 2) which well corresponded to the effect of a partial I_{K1} inhibition by 10 μM Ba^{2+} (Fig. 3). All these effects were fully reversible during the subsequent wash-out.

4.1. Dual aminophylline effect

A dual aminophylline effect on I_{K1} was observed in our previous study on rat ventricular myocytes (in 4 out of 12 rat cardiomyocytes, Fig. 4C, lower panel; [30]). The results presented here showed the activation effect less frequently - it was observed in only 1 out of 10 porcine cardiomyocytes (Figs. 1D and 2C, lower panel) and in none of 8 human cardiomyocytes at 30 μM aminophylline (Fig. 4C, upper panel). It might be a consequence of *e.g.* interspecies differences or differences in the used temperature (37 °C in porcine and human *vs.* 23 °C in rat cardiomyocytes). The origin of the dual aminophylline effect and its clinical significance have been thoroughly analyzed and discussed in our recently published paper [30].

4.2. Impact of separated aminophylline-induced I_{K1} inhibition on AP morphology

As shown in Fig. 5, the average inhibitory effect of 100 μM aminophylline on I_{K1} resulted in a substantial increase of APD_{50} as well

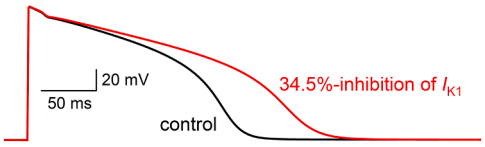

Fig. 5. Impact of I_{K1} inhibition on the action potential (AP) repolarization as simulated in a previously published porcine ventricular cell model ([12]; I_{Ca}, I_{Kr}, and I_{Ks} were suppressed during these simulations in agreement with their block within experiments). The control AP waveform (the black line) was substantially prolonged when the average I_{K1} inhibition by 34.5 % induced by 100 μM aminophylline in the experiments was introduced into the model (the red line). The simulated effect agreed well with the average effect observed during AP measurements in porcine ventricular myocytes (Fig. 2).

as APD_{90} (by ~30 and 34 %, respectively) in a mathematical model of porcine ventricular myocyte (previously published by [12]) under the same conditions that were used in our experiments (including a complete inhibition of I_{Ca}, I_{Kr}, and I_{Ks} to uncover AP changes resulting from the aminophylline-induced effect on I_{K1}). This result of mathematical modelling agrees well with the measured data (APD_{50} and APD_{90} were prolonged by ~35 and 33 %, respectively; Figs. 2A and 2B). Moreover, Ba^{2+} at the concentration of 10 μM, which causes partial inhibition of I_{K1} comparable to 100 μM aminophylline, resulted in similar AP changes (Fig. 3). This suggests that the experimentally observed delay in cardiac cell repolarization under complete inhibition of I_{Ca}, $I_{Kr,}$ and I_{Ks} might be a consequence of the inhibition of I_{K1} alone and that, except for I_{K1} and possibly I_{Ca}, I_{Kr}, and I_{Ks}, no other ionic membrane currents should be sensitive to 100 μM aminophylline. The surprisingly high impact of I_{K1} inhibition on APD appears to result from a different contribution of I_{K1} and I_{Kr} to AP repolarization in porcine ventricular myocytes *versus* that observed in human cardiomyocytes [12].

Considering the impact of aminophylline on I_{K1}, changes in RMP may be expected. However, no significant aminophylline-induced changes were apparent in the case of RMP (-69.8 ± 3.1 mV in control *vs.* −67.6 ± 2.8 mV under 100 μM aminophylline; $n = 5$, $P > 0.05$; Fig. 2B). Under the effect of 10 μM Ba^{2+} (Fig. 3), a significant depolarizing shift of RMP was observed, however, the change was tiny (by 2.7 %, from −74.2 ± 1.4 to −72.2 ± 1.4 mV, $n = 7$, $P < 0.05$), similar to that observed under 100 μM aminophylline (by 3.2 %), suggesting a consistent action of both drugs on I_{K1} in these concentrations.

4.3. Clinical relevance

Kir channels play an important role in various pathologies (*e.g.* [7,36, 32,39]) including arrhythmias (for a review, see [3]). Hence, drug-induced alterations in the function of Kir channels including those responsible for I_{K1} (*e.g.* [29,10,21,16]) may contribute to cardiac side effects of various primarily non-cardiac agents.

The average inhibitory effect of the bronchodilator aminophylline on I_{K1} increased with its increasing concentrations between 10 and 100 μM, reaching ~40 and ~20 % at 100 μM in healthy porcine and failing human ventricular myocytes, respectively, at −50 mV (Fig. 4). These concentrations cover well the common therapeutic plasma concentration of the drug [35]. In clinical practice, even a higher effect of aminophylline on I_{K1} might be observed because several times higher toxic levels of the drug have been reported in the case of overdose [15, 26].

The average inhibitory effect of aminophylline on I_{K1} and consequent delay in cardiac repolarization (Figs. 1 and 2, respectively) might result in a prolongation of QT interval and formation of early afterdepolarizations (EADs; [11,9,38]). The large scatter in the effects of aminophylline on individual cells in all tested species and even activation of I_{K1} that we observed in both porcine and rat ventricular myocytes (Fig. 4C, middle and lower panels) might increase the heterogeneity in the repolarization process (see Fig. 2C) and lead to the formation of premature ventricular complexes even in the absence of EADs [38]. However, as later discussed in the Limitations of the study, we examined the aminophylline effect on a single-cell level, and changes in AP waveform were investigated in the absence of several pivotal ionic currents, namely I_{Ca}, I_{Kr}, and I_{Ks}, which both limit considerations of the real effect of aminophylline on cardiac electrophysiology in the clinical setting, encouraging its further testing.

Since PDE inhibition is considered the main effect of aminophylline, an accumulation of cAMP, activation of protein kinase A (PKA), and associated changes in cardiac ionic currents, similar to those observed under β-adrenergic stimulation, might be expected, even in the absence of a direct interaction of aminophylline with the channels. These include the currents which were inhibited in the course of the majority of our experiments. Both I_{Ca} and I_{Ks} are well known to be activated by the cAMP-PKA pathway [18,2,27], thus, aminophylline might cause their increase which would have opposing effects on AP repolarization, leading to its delay in the case of I_{Ca} stimulation whereas in its acceleration in the case of I_{Ks} stimulation. In contrast, I_{Kr} was slightly decreased under β-adrenergic stimulation which diminished its role in cardiac cell repolarization in both guinea pig cardiomyocytes and the human heart [18,2]. Hence, in addition to the possible direct effects, an indirect effect of aminophylline, particularly on I_{Ca} and I_{Ks}, should be investigated in the future to resolve the apparent ambiguities.

4.4. Limitations of the study

Several potential limitations of this study should be considered. Here, we exclusively focused on the effect of aminophylline on I_{K1}. However, other ionic currents active during cardiac AP plateau and repolarization (*e.g.* I_{Ca}, I_{Kr}, and I_{Ks} or late sodium current $I_{Na,late}$) may be either directly or indirectly (through cAMP-PKA cascade) affected by the aminophylline action. Therefore, further investigation is needed to bring more insight into the complex effect of this clinically important agent.

The impact of aminophylline-induced I_{K1} changes on AP morphology very likely differs in porcine and human cardiomyocytes. As suggested by Gaur et al. [12] and as known from experimental studies (*e.g.* [1]), AP repolarization in human ventricular myocytes is not very sensitive to I_{K1} changes in comparison to that in porcine cardiomyocytes [12]. Hence, the real impact of aminophylline may differ in the human heart and should be further investigated, best in both healthy and diseased human cardiomyocytes where the contribution of individual ionic currents to AP repolarization is altered.

In this study, experiments were performed by the whole cell patch clamp technique in single, enzymatically isolated cells. To bring the data nearer to the clinical practice, recordings from multicellular specimens or even from the whole heart, at various pacing frequencies, and even during restitution pacing protocols might be beneficial and should be a part of future studies.

5. Conclusions

To sum up the main results of this study, we first tested the effect of clinically relevant concentrations of aminophylline on I_{K1} in healthy porcine and failing human ventricular myocytes at 37°C. A comparable average inhibitory effect was observed in both species resulting in a significant delay of repolarization in porcine ventricular myocytes under the same experimental conditions, *i.e.* during I_{Ca}, I_{Kr}, and I_{Ks} inhibition, which enabled separation of the impact of aminophylline-induced I_{K1}-changes on AP waveform. Moreover, a large scatter of the aminophylline effect on I_{K1} including even I_{K1} activation was apparent. The resulting heterogeneity in cardiac repolarization induced by aminophylline at clinically relevant concentrations might contribute to the arrhythmogenesis observed during the use of aminophylline in clinical practice. Since this study was specifically focused on the effect of aminophylline on I_{K1} channels, the effects of aminophylline on other ionic membrane currents and their proarrhythmic consequences should be addressed in

the future.

CRediT authorship contribution statement

Olga Švecová: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Markéta Bébarová:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Michal Pásek:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Roman Kula:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Petr Fila:** Writing – review & editing, Writing – original draft, Methodology. **Edita Jeklová:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition. **Martin Pešl:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

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Data Availability

Data will be made available on request.

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