

# Altered K<sup>+</sup> current profiles underlie cardiac action potential shortening in hyperkalemia and $\beta$ -adrenergic stimulation

Bence Hegyi, Ye Chen-Izu, Leighton T. Izu, and Tamás Bányász

**Abstract:** Hyperkalemia is known to develop in various conditions including vigorous physical exercise. In the heart, hyperkalemia is associated with action potential (AP) shortening that was attributed to altered gating of K<sup>+</sup> channels. However, it remains unknown how hyperkalemia changes the profiles of each K<sup>+</sup> current under a cardiac AP. Therefore, we recorded the major K<sup>+</sup> currents (inward rectifier K<sup>+</sup> current,  $I_{K1}$ ; rapid and slow delayed rectifier K<sup>+</sup> currents,  $I_{Kr}$  and  $I_{Ks}$ , respectively) using AP-clamp in rabbit ventricular myocytes. As K<sup>+</sup> may accumulate at rapid heart rates during sympathetic stimulation, we also examined the effect of isoproterenol on these K<sup>+</sup> currents. We found that  $I_{K1}$  was significantly increased in hyperkalemia, whereas the reduction of driving force for K<sup>+</sup> efflux dominated over the altered channel gating in case of  $I_{Kr}$  and  $I_{Ks}$ . Overall, the markedly increased  $I_{K1}$  in hyperkalemia overcame the relative decreases of  $I_{Kr}$  and  $I_{Ks}$  during AP, resulting in an increased net repolarizing current during AP phase 3.  $\beta$ -Adrenergic stimulation of  $I_{Ks}$  also provided further repolarizing power during sympathetic activation, although hyperkalemia limited  $I_{Ks}$  upregulation. These results indicate that facilitation of  $I_{K1}$  in hyperkalemia and  $\beta$ -adrenergic stimulation of  $I_{Ks}$  represent important compensatory mechanisms against AP prolongation and arrhythmia susceptibility.

**Key words:** hyperkalemia, sympathetic stimulation, cellular electrophysiology, heart, potassium channels, action potential voltage-clamp, physical exercise, arrhythmia.

**Résumé :** On sait que l'hyperkaliémie se produit dans diverses situations, y compris pendant l'exercice physique vigoureux. Dans le cœur, l'hyperkaliémie est associée avec une diminution de la durée du potentiel d'action (PA), qui est attribuée à des canaux K<sup>+</sup> dont les propriétés de « gating » sont altérées. Toutefois, on ne sait toujours pas comment l'hyperkaliémie entraîne des variations dans le profil de chacun des courants K<sup>+</sup> à la base du PA cardiaque. Par conséquent, nous avons enregistré les principaux courants K<sup>+</sup> (courant à rectification entrante ( $I_{K1}$ ); courants à rectification rapide et lente ( $I_{Kr}$  et  $I_{Ks}$ , respectivement)) à l'aide de la technique de clamping du PA dans des myocytes ventriculaires de lapin. Comme les ions K<sup>+</sup> peuvent s'accumuler à des fréquences cardiaques élevées pendant une stimulation sympathique, nous avons aussi étudié l'effet de l'isoprotérénol sur ces courants K<sup>+</sup>. Nous avons observé qu' $I_{K1}$  était nettement augmenté en hyperkaliémie, tandis que la diminution de la force motrice de l'efflux de K<sup>+</sup> dominait comparativement au défaut de « gating » des canaux dans le cas d' $I_{Kr}$  et d' $I_{Ks}$ . Dans l'ensemble, l'augmentation marquée d' $I_{K1}$  en hyperkaliémie parvenait à contrer la diminution relative d' $I_{Kr}$  et d' $I_{Ks}$  pendant le PA, entraînant une augmentation nette des courants de repolarisation pendant la phase 3 du PA. La stimulation  $\beta$ -adrénergique d' $I_{Ks}$  fournissait aussi une puissance de repolarisation supplémentaire pendant l'activation sympathique, même si l'hyperkaliémie limitait la régulation à la hausse d' $I_{Ks}$ . Ces résultats montrent que la facilitation d' $I_{K1}$  en hyperkaliémie et la stimulation  $\beta$ -adrénergique d' $I_{Ks}$  représentent des modes d'action compensatoires importants contre l'augmentation de la durée du PA et la susceptibilité aux arythmies. [Traduit par la Rédaction]

**Mots-clés :** hyperkaliémie, stimulation sympathique, électrophysiologie cellulaire, cœur, canaux potassiques, voltage-clamp du potentiel d'action, exercice physique, arythmie.

## Introduction

Hyperkalemia is commonly encountered clinically in various physiological and pathological conditions including vigorous physical exercise, ischemia, kidney failure, hemolysis, endocrine diseases, and side effects of the pharmacological therapy (Weiss et al. 2017). Muscle contraction has been shown to cause release of intracellular K<sup>+</sup> that may lead to marked hyperkalemia especially during strenuous exercise. As a consequence of that, K<sup>+</sup> concentration in blood plasma may rise up to 8–9 mM during intense physical activity in humans (Hansen et al. 2005; Medbo and

Sejersted 1990; Tenan et al. 2010). This value is about double the normal extracellular K<sup>+</sup> concentration ( $[K^+]_o$ ), thus it significantly reduces the Nernst potential for K<sup>+</sup>. However, despite the reduction of driving force for K<sup>+</sup> efflux that predicts a lengthening of the action potential (AP), hyperkalemia has been shown to shorten cardiac AP (Weidmann 1956). However, hyperkalemia is known to depolarize the cell membrane that alters Na<sup>+</sup> and Ca<sup>2+</sup> channel availability (steady-state inactivation) and recovery from inactivation influencing cardiac excitability and excitation-contraction coupling (Hansen et al. 2005; Sejersted and Sjøgaard

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2000). Moreover, an increase in incidence of atrial fibrillation and ventricular arrhythmias during intense physical activity has been reported (Albert et al. 2000; Manolis and Manolis 2016). Vigorous exercise, especially in untrained subjects but also in top athletes, has been associated with sudden cardiac death (Albert et al. 2000; Busuttil 1990; Varro and Bacsko 2010). Even though the pathophysiology of these fatal events is complex, electrolyte abnormalities like hyperkalemia are often listed amongst the factors leading to cardiac electrical disorders. Additionally, the existence of multiple adaptive mechanisms that preserves cellular functions in hyperkalemia underlines the severity of  $[K^+]_o$  elevation during exercise. Longitudinal studies using either animal models or human volunteers have shown an increase in muscle  $Na^+/K^+$  pump density and its transport capacity, a shift between the  $\alpha$  and  $\beta$  isoforms of  $Na^+/K^+$  pump mRNA expression levels, and a reduction of ATP-sensitive  $K^+$  channel expression as a consequence of endurance training (Gunnarsson et al. 2013; Murphy et al. 2007).

The mechanistic understanding of cellular mechanisms that serve as defense mechanisms against hyperkalemia-induced cardiac arrhythmias has high importance. High  $[K^+]_o$  is known to facilitate the inward rectifier  $K^+$  current ( $I_{K1}$ ) despite the reduced driving force for  $K^+$  efflux (Boyett et al. 1980; McAllister and Noble 1966). Moreover, it has also been reported that the rapid delayed rectifier  $K^+$  current ( $I_{Kr}$ ) but not the slow component ( $I_{Ks}$ ) exhibits a similar response to elevated  $[K^+]_o$  (Chang and Shieh 2013; Matsuda 1991; Sanguinetti et al. 1995; Sanguinetti and Jurkiewicz 1992; Scamps and Carmeliet 1989). These previous studies characterized the behavior of different  $K^+$  channels in various  $[K^+]_o$ , but did not provide information on the exact profile and contribution of  $K^+$  currents under a physiological AP. It is well established that the AP shortening seen in hyperkalemia is a direct effect of  $K^+$  ions (Weidmann 1956), but there is no information on the magnitude of individual  $K^+$  current contribution to changes in AP morphology under physiologically relevant conditions.

The goal of this work was to investigate how ventricular cardiomyocytes maintain sufficient AP repolarization when  $[K^+]_o$  increases, and driving force for  $K^+$  is reduced. To understand the mechanism of altered repolarization, we studied the effect of hyperkalemia on the profile of the major  $K^+$  currents during the ventricular AP. We recorded  $I_{Kr}$ ,  $I_{Ks}$ , and  $I_{K1}$  using AP voltage-clamp sequential dissection technique (Banyasz et al. 2011) to determine (1) what is the impact of elevated  $[K^+]_o$  on the profile of each  $K^+$  current under the AP; and (2) how the relative contribution of the  $K^+$  currents to net repolarization changes in high  $[K^+]_o$ . As physical exercise is often associated with sympathetic activation, experiments were performed also in the presence of isoproterenol (ISO). We tested the hypotheses that (1) upregulation of  $I_{K1}$  is the key step in adaptation to hyperkalemia and (2)  $\beta$ -adrenergic stimulation of  $I_{Ks}$  contributes to the increased repolarization power during sympathetic activation.

## Materials and methods

### Ethical approval

All animal handling and laboratory procedures were in accordance with the approved protocol of the local Institutional Animal Care and Use Committee at University of California, Davis (Protocol No. 18803), conforming to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (8<sup>th</sup> edition, 2011).

### Animal model and cell isolation

Sixteen adult male (3–4 months old, 2.5–3 kg) New Zealand White rabbits (Charles River, Wilmington, Massachusetts, USA) rabbits were used for experiments. Fifteen minutes before terminal surgery, rabbits were injected with heparin (400 U/kg, i.v.) and then anesthetized with isoflurane (3%–3.5%) inhalation. After achieving deep anaesthesia, hearts were quickly excised, and

placed in a cold Tyrode solution. Then, a standard enzymatic technique was used to isolate ventricular cardiomyocytes at 37 °C as previously described (Hegyi et al. 2016). Briefly, hearts were mounted on a Langendorff apparatus and retrogradely perfused for 3–5 min with oxygenated Tyrode solution to remove blood from the coronary vasculature. Then, a  $Ca^{2+}$ -free Tyrode solution was perfused for 3 min to stop the contraction of the heart. Next, a Tyrode solution supplemented with 1 mg/mL type II collagenase (Worthington, Lakewood, New Jersey, USA), 0.05 mg/mL protease type XIV (Sigma-Aldrich, St. Louis, Missouri, USA), and 50  $\mu$ M  $Ca^{2+}$  was used to perfuse the heart for 25–30 min to enzymatically dissociate cells. After perfusion, both atria and the right ventricle were removed, the left ventricle was minced, and cardiomyocytes were then harvested and stored in a modified bicarbonate-containing Tyrode solution (BTY) with the following composition (in mM): NaCl 124,  $NaHCO_3$  25, KCl 4,  $CaCl_2$  1.2,  $MgCl_2$  1, HEPES 10, glucose 10; pH 7.4 (adjusted using NaOH) and osmolality of 295–300 mOsm/kg.

### Electrophysiology

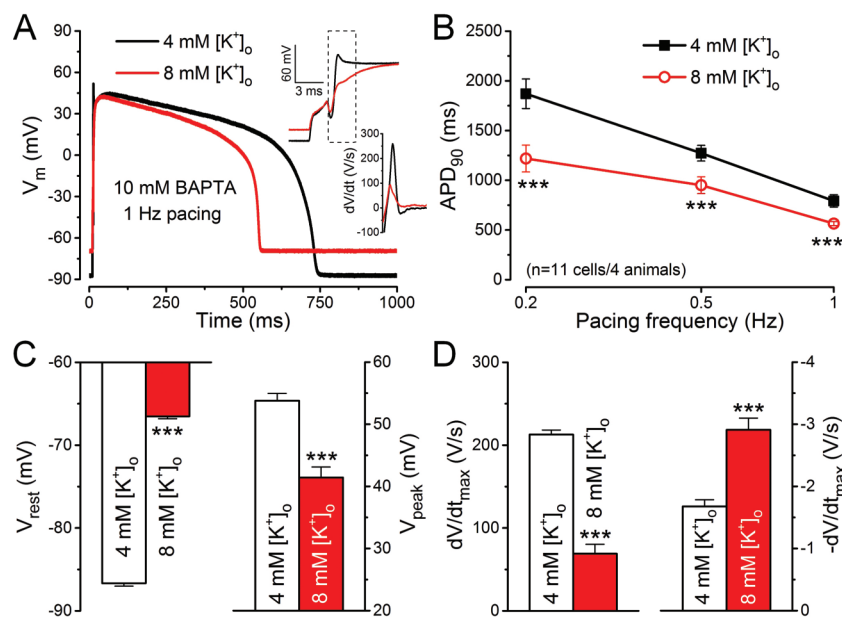
Isolated ventricular cardiomyocytes were placed in a temperature-controlled plexiglass chamber (Cell Microsystems Inc., Research Triangle Park, North Carolina, USA), then continuously perfused with BTY solution (see composition above). The internal solution contained the following (in mM): K-Aspartate 100, KCl 25, NaCl 5, Mg-ATP 3, HEPES 10, cAMP 0.002, phosphocreatine dipotassium salt 10, and BAPTA 10; pH 7.2 (i.e., the intracellular  $Ca^{2+}$  concentration was buffered to nominally zero). Electrodes were fabricated from borosilicate glass (World Precision Instruments Inc., Sarasota, Florida, USA) having tip resistances of 2–2.5 M $\Omega$  when filled with internal solution. The electrodes were connected to the input of an Axopatch 200B amplifier (Axon Instruments, Union City, California, USA). Outputs from the amplifier were digitized at 50 kHz using Digidata1440A A/D card (Molecular Devices, San Jose, California, USA) under software control (pClamp 10). Seal formation was performed always in BTY with 4 mM  $[K^+]_o$ . The series resistance was typically 3–5 M $\Omega$ , and it was compensated by 85%. The seal condition was monitored periodically, and experiments were discarded from analysis if the series resistance increased by >10%. All experiments were conducted at  $36 \pm 0.1$  °C.

APs of single ventricular cardiomyocytes were recorded in ruptured patch current-clamp experiments. The cells were paced using an external stimulator (Grass S44; Grass Instrument Co., Quincy, Massachusetts, USA) generating depolarizing pulses (2 ms long with 1.5 $\times$  the threshold amplitude) delivered via the patch pipette at a steady-state frequency of 0.2, 0.5, and 1 Hz. Experiments have been performed using the internal solution described above (containing 10 mM BAPTA) and the extracellular BTY solution with 4 mM  $[K^+]_o$ , then perfusion was switched to BTY with 8 mM  $[K^+]_o$  for 3 min, and the recordings were repeated in the same cell.

$I_{K1}$  was measured in conventional voltage-clamp using a ramp protocol arising from –160 mV to 30 mV within 300 ms using a holding potential of –80 mV. Extracellular BTY solution was supplemented with 1  $\mu$ M E-4031, 1  $\mu$ M HMR-1556, 10  $\mu$ M nifedipine, and 10  $\mu$ M tetrodotoxin to inhibit  $I_{Kr}$ ,  $I_{Ks}$ ,  $Ca^{2+}$  and  $Na^+$  currents, respectively. After recording the current of interest in 4 and 8 mM  $[K^+]_o$ , 100  $\mu$ M  $Ba^{2+}$  was added to inhibit  $I_{K1}$ .

AP-clamp experiments were conducted as previously described (Chen-Izu et al. 2017; Hegyi et al. 2018b). Briefly, the basic steps included the following: (1) Apply cell's own steady-state AP (self AP-clamp) or choose a previously recorded typical AP (canonical AP-clamp) onto the cell as voltage command under V-clamp at a given pacing frequency. The net current output (reference current) should reach steady-state and be stable over time. (2) Isolate the current of interest by using its specific inhibitor to remove it from the net current output (compensation current). (3) The current of interest is then obtained by subtraction (drug-sensitive

**Fig. 1.** Shortening of ventricular action potential (AP) in hyperkalemia. (A) Representative ventricular APs at 1 Hz pacing in either 4 or 8 mM  $[K^+]_o$ . Pipette solution contained 10 mM BAPTA. AP upstroke and its derivative ( $dV/dt$ ) are enlarged in insets. (B) AP duration measured at 90% repolarization ( $APD_{90}$ ) was significantly decreased in hyperkalemia in a reverse-rate dependent manner. (C) Depolarization of resting membrane potential ( $V_{rest}$ ) followed the change in Nernst-potential for  $K^+$ , which led to a decrease in peak voltage of the AP overshoot ( $V_{peak}$ ). (D) Maximal upstroke velocity ( $dV/dt_{max}$ ) significantly decreased, while maximal rate of repolarization ( $-dV/dt_{max}$ ) significantly increased in hyperkalemia. Columns/symbols and bars represent mean  $\pm$  SEM.  $n = 11$  cells from 4 animals. Student's paired  $t$  test. \*\*\*,  $p < 0.001$ . [Color online.]



current = reference current – compensation current). (4) Next, isolate the second current of interest by applying the second channel blocker; when it reaches steady state, another compensation current is recorded, and the second current of interest can be determined again by subtraction. (5) Repeating the same procedure, a third (or more) ionic current can be determined by sequentially applying the specific inhibitors for each ion channel in a cumulative manner and by obtaining the difference currents. Representative traces are shown in the Supplementary Data<sup>1</sup> with further methodological details. A prerecorded, typical, physiological rabbit ventricular AP (canonical AP-clamp) was used at 2 Hz frequency (Hegyi et al. 2015, 2018b). This typical AP has been selected from our collection of APs recorded at 2 Hz pacing frequency in extracellular BTY solution and an internal solution with the following composition (in mM): K-aspartate 110, KCl 25, NaCl 5, Mg-ATP 3, HEPES 10, cAMP 0.002, phosphocreatine dipotassium salt 10, and EGTA 0.01; pH 7.2. This ionic composition preserves physiological  $Ca^{2+}$  transient and myocyte contraction (Horvath et al. 2013). However, in the present study, all AP-clamp experiments have been performed using 10 mM BAPTA in the pipette solution and the extracellular BTY solution was supplemented with 10  $\mu$ M nifedipine and 10  $\mu$ M tetrodotoxin to avoid contamination of the measured  $K^+$  currents with  $Na^+/Ca^{2+}$  exchanger, L-type  $Ca^{2+}$  and  $Na^+$  currents, respectively (Hegyi et al. 2018b). Under AP-clamp, all ionic currents were recorded as difference currents after each specific blocker had reached its steady-state effect (2 min of perfusion). Sixty consecutive traces were recorded (to evaluate the stability) and averaged in each case before applying a drug (reference current) and 2 min after drug application (compensation current). The following sequence of drugs was used to measure the major  $K^+$  currents during phase 3 repolarization of the AP: 1  $\mu$ M HMR-1556 for  $I_{Ks}$  (Thomas et al. 2003), 1  $\mu$ M E-4031 for  $I_{Kr}$  (Wettwer et al. 1991), and 100  $\mu$ M  $Ba^{2+}$  for  $I_{K1}$  (Alagum

et al. 2001). When studying the effect of  $\beta$ -adrenergic stimulation, 10 nM ISO was added to the perfusion solution and after it reached a steady-state effect (3 min), the  $K^+$  current inhibitors were added to the perfusion solution in a cumulative manner. Experiments were excluded from analysis if membrane current did not reach steady-state.

Ionic currents were normalized to cell capacitance, determined in each cell using short (10 ms) hyperpolarizing pulses from  $-10$  to  $-20$  mV. Cell capacitance was  $140.58 \pm 1.26$  pF ( $n = 74$  cells/16 animals).

Chemicals and reagents were purchased from Sigma-Aldrich if not specified otherwise. E-4031 and HMR-1556 were from Tocris Bioscience (Bristol, UK).

### Statistical analysis

Data are expressed as mean  $\pm$  SEM. The number of cells in each experimental group was reported as  $n$  = number of cells/number of animals. Statistical significance of differences was evaluated using Student's paired  $t$  test and ANOVA with Bonferroni correction as appropriate. Differences were deemed significant if  $p < 0.05$ .

## Results

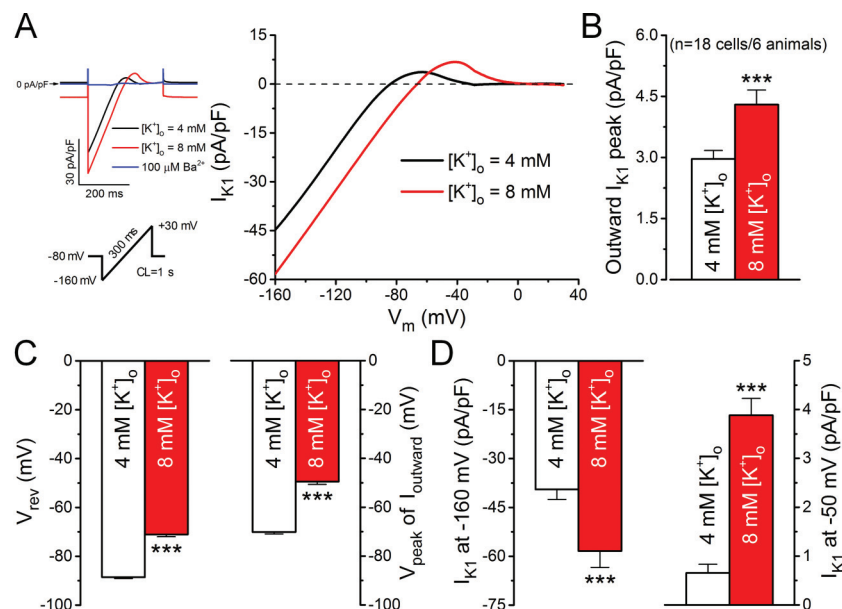
### Hyperkalemia shortens AP duration and increases the rate of repolarization

First, we measured APs and characterized the frequency-dependent changes in 8 mM  $[K^+]_o$  (8K) vs. 4 mM  $[K^+]_o$  (4K). All experiments were performed in the presence of 10 mM BAPTA in the pipette solution to avoid the effect of secondary changes in  $[Ca^{2+}]_i$ . As shown in representative APs in Fig. 1A, substantial depolarization and AP shortening was observed in 8K. AP duration measured at 90% repolarization ( $APD_{90}$ ) was significantly shortened in 8K vs. 4K. The difference in  $APD_{90}$  was smaller at high

<sup>1</sup>Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjpp-2019-0056>.



**Fig. 2.** Outward  $I_{K1}$  is significantly increased in hyperkalemia. (A) Representative traces and  $I$ - $V$  relationships of 100  $\mu$ M  $Ba^{2+}$ -sensitive inward rectifier  $K^+$  current ( $I_{K1}$ ) in a rabbit ventricular myocyte (applied voltage protocol is shown in the inset).  $[Ca^{2+}]_i$  was buffered to nominally zero by 10 mM BAPTA in the pipette solution.  $I_{Kr}$ ,  $I_{Ks}$ , L-type  $Ca^{2+}$ , and voltage-gated  $Na^+$  currents were inhibited using 1  $\mu$ M E-4031, 1  $\mu$ M HMR-1556, 10  $\mu$ M nifedipine, and 10  $\mu$ M tetrodotoxin, respectively. (B) Hyperkalemia increased the peak outward current. (C) The shift in reversal potential followed the change in the Nernst-potential for  $K^+$ . The voltage where the outward current reached its peak density was more positive in high  $[K^+]_o$ . (D) Both inward and outward  $I_{K1}$  (measured at  $-160$  and  $-50$  mV, respectively) were significantly increased in hyperkalemia. Columns and bars represent mean  $\pm$  SEM.  $n = 18$  cells from 6 animals. Student's paired  $t$  test. \*\*\*,  $p < 0.001$ . [Color online.]



pace rates and the frequency response of  $APD_{90}$  was less prominent in 8K (Fig. 1B). Resting membrane potential ( $V_{rest}$ ) was more positive in line with the change in Nernst-potential for  $K^+$  (Fig. 1C). Accordingly, the AP peak voltage ( $V_{peak}$ ) and maximal upstroke velocity ( $dV/dt_{max}$ ) significantly decreased in 8K, consistent with the decreased availability of  $Na^+$  channels at depolarized  $V_{rest}$ . But importantly, the maximum rate of repolarization ( $-dV/dt_{max}$ ) increased by 75% in 8K (Fig. 1D).

### Hyperkalemia increases outward $I_{K1}$

Next, we examined the mechanism of  $I_{K1}$  facilitation shown in Fig. 2. In the following experiments,  $I_{K1}$  was measured as  $Ba^{2+}$ -sensitive current under a slow voltage ramp (Fig. 2A). Hyperkalemia shifted the reversal potential of the measured current to more positive values as expected from the change in Nernst-potential for  $K^+$ . However, despite the reduced driving force for  $K^+$ , 8K markedly increased outward  $I_{K1}$ . The peak outward  $I_{K1}$  density was increased by 45% in 8K vs. 4K ( $3.00 \pm 0.08$  pA/pF vs.  $4.32 \pm 0.12$  pA/pF) as shown in Fig. 2B. The reversal potential and the membrane potential where  $I_{K1}$  peaked were shifted by  $17.52 \pm 1.04$  and  $20.98 \pm 0.85$  mV, respectively. The increased inward  $I_{K1}$  followed the shift of Nernst-potential for  $K^+$  (Figs. 2C, 2D) and the slope of the inward  $I_{K1}$  did not change in 8K vs. 4K. In contrast to the inward  $I_{K1}$ , the outward  $I_{K1}$  was markedly enhanced in 8K when determined at  $-50$  mV (Fig. 2D).

### Profile of major repolarizing $K^+$ currents under ventricular AP in hyperkalemia and $\beta$ -adrenergic stimulation

Next, we compared the profile of  $I_{K1}$ ,  $I_{Kr}$ , and  $I_{Ks}$  currents under AP-clamp in 4K and 8K at 2 Hz pacing rate under AP-clamp in the same cell. To eliminate the impact of individual cell's AP configuration on the profiles of  $K^+$  currents studied, we used the same prerecorded, typical, physiological rabbit AP (obtained with preserved  $[Ca^{2+}]_i$  transient) in all AP-clamp experiments. Because in this study we were interested in the profiles of each  $K^+$  current under AP-clamp, inward currents ( $Na^+$ ,  $Ca^{2+}$ , and  $Na^+/Ca^{2+}$  exchanger currents) were inhibited using tetrodotoxin and nifedipine in the extracellular solution as well as 10 mM BAPTA in the internal (pipette) solution.

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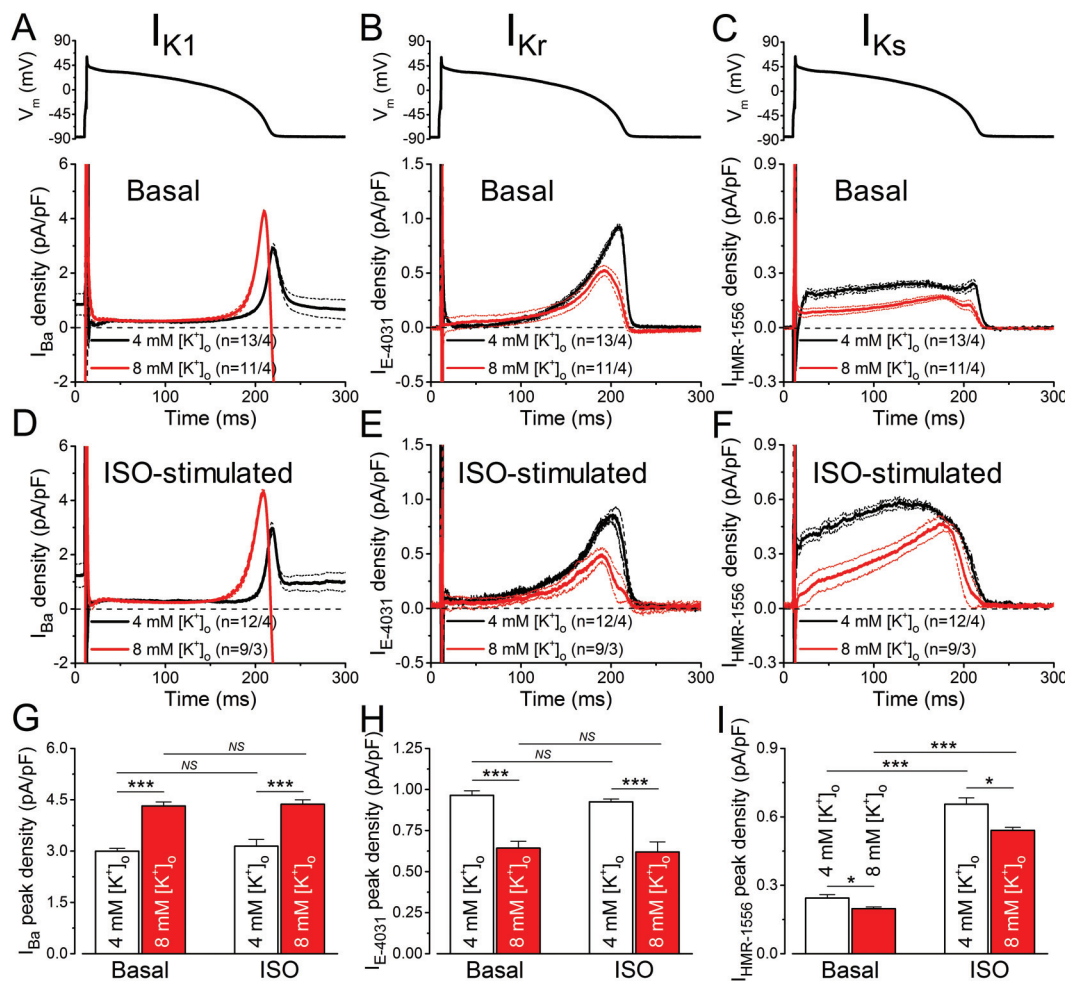
$I_{K1}$  (measured as  $Ba^{2+}$ -sensitive current) was markedly increased under AP-clamp in 8K vs. 4K as shown in Fig. 3A.  $I_{K1}$  reached higher peak at earlier time, i.e., at more positive membrane potential. This increased current provided the extra repolarizing power that facilitated AP repolarization shown in Fig. 1C. Moreover, the positive shift in Nernst-potential for  $K^+$  resulted in a large inward current at diastolic potential of  $-86$  mV ( $0.81 \pm 0.39$  pA/pF vs.  $-9.19 \pm 1.34$  pA/pF in 4K and 8K, respectively).  $I_{Kr}$  (measured as E-4031-sensitive current) started to increase during phase 2 and peaked during phase 3 of the AP (Fig. 3B). The peak  $I_{Kr}$  density was reduced from  $0.96 \pm 0.03$  pA/pF in 4K to  $0.64 \pm 0.04$  pA/pF in 8K; however, the profile of  $I_{Kr}$  under AP-clamp did not change significantly. Baseline  $I_{Ks}$  (measured as HMR-1556-sensitive current) was a small current under AP plateau, and it was further decreased in 8K vs. 4K ( $0.24 \pm 0.02$  pA/pF vs.  $0.19 \pm 0.01$  pA/pF; Fig. 3C).

$\beta$ -Adrenergic receptor stimulation using 10 nM ISO did not affect  $I_{K1}$  significantly in either 4K or 8K when  $[Ca^{2+}]_i$  was buffered with 10 mM BAPTA in the pipette (Fig. 3D).  $I_{Kr}$  also did not change after ISO stimulation (Fig. 3E). On the contrary,  $I_{Ks}$  was significantly upregulated upon ISO application both in 4K and in 8K (Fig. 3F) and significant  $I_{Ks}$  was observed during the early AP plateau that may reflect both faster activation of  $I_{Ks}$  and accumulation of the open state channels due to the slow deactivation kinetics of  $I_{Ks}$ , as previously reported (Rocchetti et al. 2006). Interestingly, 8K not simply reduced the magnitude of  $I_{Ks}$  in ISO, but markedly distorted the shape of the current. The profile of  $I_{Ks}$  under AP-clamp in 8K following ISO stimulation became similar to that of  $I_{Kr}$ . Statistical analysis of the currents revealed that  $I_{K1}$  outward peak density increased by  $\sim 40\%$  in 8K,  $I_{Kr}$  peak decreased by  $\sim 30\%$ , while  $I_{Ks}$  decreased by  $\sim 20\%$  (Figs. 3G–3I).

### Relative contribution of each $K^+$ current under ventricular AP in hyperkalemia and $\beta$ -adrenergic stimulation

Hyperkalemia suppressed the moving charge of  $I_{Kr}$  and  $I_{Ks}$  significantly both in control and following ISO stimulation. The re-

**Fig. 3.** Altered  $K^+$  current profiles under action potential (AP)-clamp in hyperkalemia and  $\beta$ -adrenergic stimulation. (A)  $I_{K1}$  traces (mean  $\pm$  SEM) recorded under AP-clamp in physiological (4 mM) and elevated (8 mM)  $[K^+]_o$ . A prerecorded, typical rabbit ventricular AP (shown above) was used as voltage command in all AP-clamp experiments (canonical AP-clamp) at 2 Hz pacing frequency.  $I_{K1}$  was measured as 100  $\mu$ M  $Ba^{2+}$ -sensitive current.  $[Ca^{2+}]_i$  was buffered using 10 mM BAPTA in the pipette, whereas  $Ca^{2+}$  and  $Na^+$  channels were inhibited using 10  $\mu$ M nifedipine and 10  $\mu$ M tetrodotoxin, respectively. Diastolic  $I_{K1}$  in 8 mM  $[K^+]_o$  is out of range. (B)  $I_{Kr}$  traces (mean  $\pm$  SEM) were recorded under AP-clamp using 1  $\mu$ M E-4031. (C)  $I_{Ks}$  traces (mean  $\pm$  SEM) recorded under AP-clamp using 1  $\mu$ M HMR-1556. (D–F)  $I_{K1}$ ,  $I_{Kr}$ , and  $I_{Ks}$  traces recorded (mean  $\pm$  SEM) in the presence of 10 nM isoproterenol (ISO) in 4 and 8 mM  $[K^+]_o$ . (G–I) Peak current densities.  $I_{K1}$  peak density was significantly increased in 8 mM  $[K^+]_o$  both in basal conditions and following ISO stimulation.  $I_{Kr}$  was decreased in 8 mM  $[K^+]_o$  and its density was unchanged by ISO stimulation.  $I_{Ks}$  was significantly upregulated following ISO stimulation; however, the increase in  $I_{Ks}$  amplitude was attenuated in 8 mM  $[K^+]_o$ . Columns and bars represent mean  $\pm$  SEM.  $n$  refers to cells/animals measured in each group. Two-way ANOVA with Bonferroni post hoc test. \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ ; NS, not significant. [Color online.]



duction of  $I_{Kr}$  under AP-clamp was similar in control and 10 nM ISO. However, 8K reduced  $I_{Ks}$  by 24% and 42% in control and 10 nM ISO, respectively. Nonetheless, the total charge carried by  $I_{Ks}$  in 10 nM ISO and in 8K was increased to 194% of that measured in 4K and in basal condition. 8K increased  $I_{K1}$  in both control and 10 nM ISO in a similar extent compared with that measured in 4K (Fig. 4A). These data indicate that  $I_{Ks}$  can compensate the hyperkalemia-induced reduction in repolarizing power of  $I_{Kr}$  only following sympathetic activation. However,  $I_{K1}$  is still able to provide considerable extra repolarizing power in the absence of sympathetic stimulation.

As repolarizing  $K^+$  currents have different profiles under AP, we analysed the relative contribution of these currents to the net repolarizing current at different AP voltages (20, -20, and -60 mV). Despite the strong rectification of  $I_{K1}$  at potentials positive to the resting potential,  $I_{K1}$  was still the largest repolarizing current during the phase 3 of the AP.  $I_{Kr}$  also significantly contributed to phase 3 repolarization (Fig. 4B). Even though  $I_{Kr}$  was diminished in

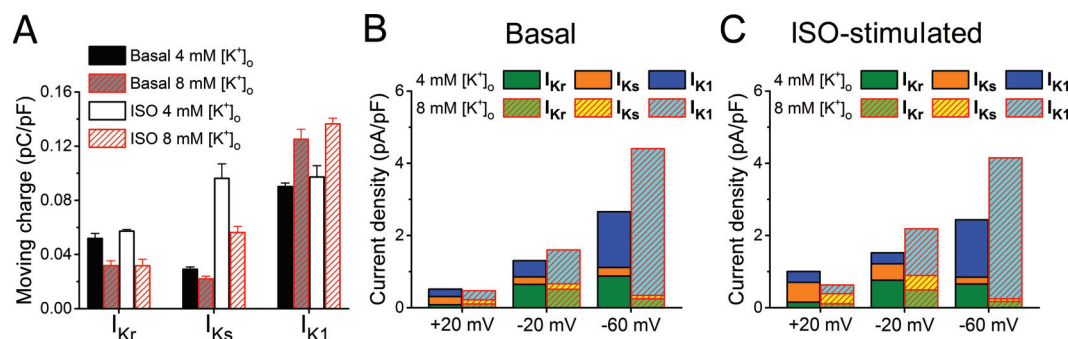
8K, this reduction was not only compensated by the  $I_{K1}$  facilitation, but the sum of the repolarizing  $K^+$  currents was increased by 65% at this phase of repolarization (Fig. 4B).  $I_{K1}$  and  $I_{Kr}$  provided almost entirely the repolarizing power during phase 3 of the AP (at -20 and -60 mV) under basal condition; however, the relative contribution of  $I_{Ks}$  to the net repolarization was markedly increased following  $\beta$ -adrenergic stimulation especially during the AP plateau phase (at 20 mV; Fig. 4C). Similar to that seen in control, hyperkalemia reduced  $I_{Ks}$  in 10 nM ISO, but the absolute magnitude of  $I_{Ks}$  was still higher than that measured in control under AP-clamp (Figs. 4B, 4C). These data indicate that  $I_{Ks}$  becomes an important contributor of the defense mechanism against hyperkalemia during sympathetic stimulation.

## Discussion

### Effect of hyperkalemia on $K^+$ currents

The goal of this project was to gain mechanistic insight on the electrophysiological adaptation of ventricular cardiomyocytes to

**Fig. 4.** Relative contributions of each  $K^+$  current in adaptation to hyperkalemia and  $\beta$ -adrenergic stimulation. (A) Net charges of  $I_{Kr}$ ,  $I_{Ks}$ , and  $I_{K1}$  in 4 and 8 mM  $[K^+]_o$  under control and in the presence of 10 nM isoproterenol (ISO). (B) Relative contributions and magnitudes of the main  $K^+$  currents during action potential (AP) phase 3 are compared in different phase of the repolarization process in 4 and 8 mM  $[K^+]_o$ . Upregulation of  $I_{K1}$  overcomes the decreases of  $I_{Kr}$  and  $I_{Ks}$  during AP phase 3 measured at  $-20$  and  $-60$  mV. (C)  $\beta$ -Adrenergic stimulation significantly increased net repolarizing current via  $I_{Ks}$  upregulation; however, the relative contribution of  $I_{K1}$  was still dominant in 8 mM  $[K^+]_o$  vs. 4 mM  $[K^+]_o$ . Columns represent mean current densities. [Color online.]



hyperkalemia. Here we showed that  $I_{K1}$  is markedly upregulated, but  $I_{Kr}$  and  $I_{Ks}$  are downregulated in hyperkalemia under the AP (Fig. 3). We found that the extent of  $I_{K1}$  upregulation exceeded the reduction of  $I_{Kr}$  and  $I_{Ks}$ . Therefore, despite the reduced driving force for  $K^+$  efflux in hyperkalemia, the net repolarizing current was increased (Fig. 4) and the AP duration was shortened (Fig. 1).

Facilitation of  $I_{K1}$  in elevated  $[K^+]_o$  due to increased single-channel conductance of  $K_{ir}$  channels has already been reported in previous studies employing rectangular voltage clamp protocols (Boyett et al. 1980; Liu et al. 2011; McAllister and Noble 1966).  $[K^+]_o$  also affects the rectification of  $I_{K1}$  resulting in increased outward  $I_{K1}$  density that was attributed to the competition between  $Mg^{2+}$  and  $K^+$  binding (Matsuda 1991). Our data provide the first experimental evidence to prove that these mechanisms are effectively facilitate  $I_{K1}$  under the ventricular AP (Figs. 2–3).

Increased  $[K^+]_o$  has also been reported to increase  $I_{Kr}$  in *Xenopus* oocyte expression system (Sanguinetti et al. 1995), but we found that hyperkalemia actually reduced  $I_{Kr}$  peak density under AP-clamp in rabbit. This conflicting result can be explained by the substantial differences between the experimental model and conditions used in these studies. Sanguinetti et al. used a *Xenopus* oocyte expression system, whereas we used freshly isolated adult rabbit ventricular cardiomyocytes. Besides the important differences between the compositions of extra- and intra-cellular solutions used, the voltage protocols were also conceptually different. Sanguinetti et al. recorded  $I_{Kr}$  with a conventional voltage clamp protocol, using 4 s long rectangular depolarization steps with 1–3 pulses/min pacing rate to study the activation and the deactivation of  $I_{Kr}$ . The long depolarizing and interpulse intervals are critical for the complete transition of the whole channel population to uniform states (equilibrium condition) but these conditions differ markedly from that present in beating heart. We used AP voltage-clamp technique, when a prerecorded physiological rabbit AP (200 ms long) was applied as voltage command at much higher pacing rate (120 pulses/min). Under these conditions, the channel population has no time for complete deactivation; therefore, the increasing magnitude of the current is explained predominantly by accumulation and not activation of the current (non-equilibrium condition) (Rocchetti et al. 2001). The traditional square-pulse voltage protocol used by Sanguinetti et al. is a precise biophysical approach aiming to understand the gating mechanism of individual channels, whereas our approach measures the actual current during an AP to gain functional insights on the role of each current in shaping the AP (Chen-Izu et al. 2017). Our data indicates that the decreased driving force for  $K^+$  efflux during AP overcomes the stimulatory effect of hyperkalemia on  $I_{Kr}$  gating. This is in line with a publication of Sanguinetti and Jurkiewicz

(1992)), who demonstrated that native  $I_{Kr}$  showed much lower  $[K^+]_o$ -dependence than the hERG channels in heterologous expression systems. Note that the exact physiological profile and magnitude of both  $I_{Kr}$  and  $I_{Ks}$  under AP crucially depend on the timing and the plateau voltages of the AP, which require further investigation.

#### Effect of $\beta$ -adrenergic stimulation on $K^+$ currents in hyperkalemia

Vigorous physical exercise is associated with increased cardiac output with higher sympathetic tone present. Activation of  $\beta$ -adrenergic receptors is known to upregulate L-type  $Ca^{2+}$  current (Hegyi et al. 2019b; Szentandrássy et al. 2012) and late  $Na^+$  current (Hegyi et al. 2018a), which enhance depolarization drive; however, the AP duration is still usually shortened because of the substantial  $K^+$  current upregulation (Banyasz et al. 2014; Hegyi et al. 2018b; Ruzsnavszky et al. 2014). Hence, we repeated our experiments in the presence of 10 nM ISO to investigate the impact of  $\beta$ -adrenergic stimulation on the adaptive mechanism to hyperkalemia. Our data indicate that  $I_{Kr}$  and  $I_{K1}$  were the 2 dominant repolarizing currents during AP under baseline conditions, but these currents were not significantly modulated by ISO treatment (Fig. 3). This later finding is in contrast with previous studies that reported slightly increased  $I_{Kr}$  and  $I_{K1}$  following  $\beta$ -adrenergic stimulation (Banyasz et al. 2014; Harmati et al. 2011). One possible explanation for this discrepancy can be the  $Ca^{2+}$ -dependence of these currents.  $I_{K1}$  has been shown to be increased acutely by the  $Ca^{2+}$ /calmodulin-dependent protein kinase II (Hegyi et al. 2019a; Wagner et al. 2009), which is also known to get activated during  $\beta$ -adrenergic stimulation (Hegyi et al. 2018a). Similarly,  $I_{Kr}$  can be upregulated by  $Ca^{2+}$ -dependent protein kinase C (Heath and Terrar 2000); however, others reported that protein kinase C decreased  $I_{Kr}$  (Cockerill et al. 2007). In this study, we buffered  $[Ca^{2+}]_i$  below its physiological diastolic level using 10 mM BAPTA in the pipette, which might have masked these regulations.

In line with previous reports (Banyasz et al. 2014; Rocchetti et al. 2006; Ruzsnavszky et al. 2014; Sala et al. 2018; Szentandrássy et al. 2012), 10 nM ISO markedly increased  $I_{Ks}$  peak density and moving charge to 268% and 331% of control, respectively (Fig. 3). Increasing  $[K^+]_o$  from 4 to 8 mM reduced  $I_{Ks}$  in a similar extent both in control and following ISO stimulation ( $I_{Ks}$  peak density decreased by 19% and 17%, respectively). However, following ISO stimulation, the net charge of  $I_{Ks}$  in 8K was still larger than that of  $I_{Kr}$  in any conditions measured in our study. These data suggests that both  $I_{Kr}$  and  $I_{Ks}$  are reduced under the AP in high  $[K^+]_o$  due to decreased driving force for  $K^+$  efflux, but the facilitation of  $I_{Ks}$  during increased  $\beta$ -adrenergic stimulation can compensate for the reduc-



tion of delayed rectifier  $K^+$  currents in hyperkalemia. The reversal of  $I_{Kr} - I_{Ks}$  dominant pattern of AP repolarization after  $\beta$ -adrenergic stimulation in rabbit cardiomyocytes is consistent with our previous results in guinea pig (Banyasz et al. 2014) and canine ventricular myocytes (Szentandrássy et al. 2012). Therefore, we propose that  $I_{Ks}$  is an important component of the cardiac adaptation to high  $[K^+]_o$  under physiological conditions, when strenuous physical activity is associated with increased sympathetic tone. Also, our observations indicate that sympathetic stimulation-induced  $I_{Ks}$  facilitation may provide efficient protection against electrical dysfunction in cardiac cells.

Transient outward  $K^+$  current ( $I_{to}$ ) was not studied here, but it also significantly contributes to AP repolarization and AP duration response to  $\beta$ -adrenergic stimulation (Sala et al. 2018; Szentandrássy et al. 2012). Therefore, the regulation of  $I_{to}$  by hyperkalemia and the way it influences the transmural dispersion of ventricular repolarization are important questions to be tested in the future. Moreover, it also has been demonstrated previously that functionally distinct  $Na^+$  channels in different transmural regions of the left ventricle also significantly contribute to the AP duration response to hyperkalemia (Cordeiro et al. 2008). The depolarization of the resting membrane potential in hyperkalemia reduces the availability and recovery from inactivation kinetics of  $Na^+$  and L-type  $Ca^{2+}$  channels influencing AP duration, conduction velocity (Weiss et al. 2017), but also  $Ca^{2+}$  cycling and signaling in cardiomyocytes (Bers 2008), which requires further investigation.

Importantly, under pathological conditions characterized by reduced repolarization reserve (Bebarova et al. 2017; Hegyi et al. 2018b, 2018c; Lengyel et al. 2007; Varro and Baczkó 2011), the significantly decreased  $I_{K1}$  and  $I_{Ks}$  may not be able to compensate for the reduced driving force for  $K^+$  in hyperkalemia and the risk of arrhythmias can be further increased.

## Conclusions

In this study, we characterized the role of 3 major  $K^+$  currents ( $I_{Kr}$ ,  $I_{Ks}$ , and  $I_{K1}$ ) in the adaptation of ventricular myocyte to hyperkalemia. We found that reduced driving force for  $K^+$  combined with altered ion channel gating results in a net reduction for  $I_{Kr}$  and  $I_{Ks}$  and a marked increase for  $I_{K1}$  in hyperkalemia. Furthermore, we demonstrated that increased  $I_{K1}$  in hyperkalemia is able to overcome the decreases of  $I_{Kr}$  and  $I_{Ks}$  during ventricular AP, thus increasing the net repolarizing current in phase 3 of the AP.  $\beta$ -Adrenergic receptor stimulation significantly upregulates  $I_{Ks}$ ; however, the  $I_{Ks}$  upregulation is diminished in hyperkalemia. Increased  $I_{K1}$  and  $I_{Ks}$  enhance the repolarizing power to reduce the risk of early afterdepolarizations and, hence, they represent an important defense mechanism against AP prolongation and arrhythmia susceptibility in hyperkalemia that may occur under intense physical activity. Interestingly, we found reduced  $I_{Kr}$  response to hyperkalemia in cardiomyocytes under AP-clamp, which contrasts with previous findings in heterologous expression of hERG channels. The attenuated regulation of native  $I_{Kr}$  may have significant arrhythmogenic consequences, especially when the repolarization reserve is reduced (e.g., in heart failure and long QT syndromes); therefore, the determinants of  $I_{Kr}$  gating alterations in hyperkalemia demand further investigation.

## Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

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