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Modulation of KCNQ1 alternative splicing regulates cardiac I_{Ks} and action potential repolarization

Hsiang-Chun Lee, MD, MSc^{1,2,3,4}, Yoram Rudy, PhD¹, Po-Yuan Chen, MSc⁵, Sheng-Hsiung Sheu, MD^{2,3,4}, Jan-Gowth Chang, MD⁶, and Jianmin Cui, PhD¹

¹Cardiac Bioelectricity and Arrhythmia Center, Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri, USA

²Cardiovascular Center, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung Medical University

³Graduate institute of Medicine, College of Medicine, Kaohsiung Medical University

⁴Department of Internal Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

⁵College of Electrical Engineering and Computer Science, National Cheng-Kung University, Tainan, Taiwan

⁶Department of Laboratory Medicine, China Medical University Hospital, Taichung, Taiwan

Abstract

Background— I_{Ks} channels, made of the pore-forming KCNQ1 and auxiliary KCNE1 subunits, play a key role in determining action potential duration (APD) in cardiac myocytes. The consequences of drug-induced KCNQ1 splice alteration remain unknown.

Objective—We study the modulation of KCNQ1 alternative splicing by amiloride and the consequent changes in I_{Ks} and action potentials (AP) in ventricular myocytes.

Methods—Canine endocardial, midmyocardial, and epicardial ventricular myocytes were isolated. Levels of KCNQ1a and KCNQ1b as well as a series of splicing factors were quantified by RT-PCR and Western blot. The impact of amiloride-induced alterations in KCNQ1b/total KCNQ1 ratio on AP was measured using whole-cell patch clamp with and without isoproterenol.

Results—With 50 μ mol/L amiloride for 6 hours, KCNQ1a at transcriptional and translational levels increased in midmyocardial but decreased in endo- and epicardial myocytes. Likewise, changes of splicing factors in midmyocardial were opposite to that in endo- and epicardial myocytes. In midmyocardial myocytes amiloride shortened APD and decreased isoproterenol-induced early afterdepolarizations significantly. The same amiloride-induced effects were demonstrated by using human ventricular myocyte model for action potentials simulations under β -adrenergic stimulation. Moreover, amiloride reduced the transmural dispersion of repolarization in pseudo-ECG.

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For correspondence: Jianmin Cui, 290C Whitaker Hall, One Brookings Dr., Saint Louis, MO 63130-4899, USA, Tel.: (314) 935-8896; Fax: (314) 935-7448; jcui@wustl.edu.

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CONFLICTS OF INTEREST: none

Conclusions—Amiloride regulates I_{Ks} and action potentials with transmural differences and reduces arrhythmogenicity through modulating KCNQ1 splicing. We suggested that modulation of KCNQ1 splicing may help prevent arrhythmia.

Keywords

action potential; alternative splicing; amiloride; I_{Ks} ; KCNQ1

INTRODUCTION

Ventricular repolarization is importantly dependent on I_{Ks} , and the contribution of I_{Ks} to action potential (AP) repolarization is enhanced during β -adrenergic stimulation.^{1–4} This enhancement is also reflected on the phenotype of most long QT syndrome type 1, which is caused by KCNQ1 (α -subunit of I_{Ks}) mutations, with ventricular arrhythmias mostly occurring under physical or emotional stress. Phosphorylation by PKA following β -adrenergic receptor stimulations greatly enhances I_{Ks} by increasing current amplitude, leftward shift in I-V curve, and by changing activation and deactivation kinetics.^{2,4} In large mammals, I_{Ks} also constitutes a “repolarization reserve” that compensates for compromised other repolarization currents, in particular I_{Kr} .^{3,4}

A weaker I_{Ks} contributes partially to the longer AP in midmyocardial (Mid) myocytes, and a prominent I_{Ks} enables endocardial (Endo) and epicardial (Epi) myocytes to be more resistant to early afterdepolarization (EAD) activity versus Mid.^{5,6} In the human and the canine heart, there are two splicing variants of KCNQ1. One is full-length, KCNQ1a, and the other is N-terminal truncated, KCNQ1b.^{7,8} KCNQ1b exerts a dominant negative effect due to a trafficking defect, and KCNQ1b transgenic mice manifest with pronounced QT prolongation and ventricular arrhythmias.^{7–10} The differential expression of KCNQ1 splicing variants has been suggested to be responsible for differential I_{Ks} amplitude and also for AP heterogeneity across the ventricular wall.¹⁰ However, whether splicing of KCNQ1 can be altered and how such alterations impact on cardiac arrhythmogenicity, remain unexplored.

Amiloride, a potassium-sparing diuretic for the treatment of hypertension and congestive heart failure since 1967, has multiple pharmacological actions. Our previous studies showed that splicing site selection in several genes can be altered by amiloride.^{11,12} Prolonged treatment with amiloride also exerts antiarrhythmic effect both in postinfarction dogs and in patients with ventricular tachycardia.^{13,14} However the cellular mechanisms have not been determined.

Here we study the electrophysiological effects of amiloride on ventricular myocytes, in terms of its effect on KCNQ1 splicing variants expression and I_{Ks} current densities, and investigate the impact of KCNQ1 splicing on the APD, isoproterenol (ISO)-induced EAD and cardiac transmural dispersion of repolarization (TDR). The mechanism of how amiloride affect the arrhythmogenicity will also be addressed.

METHODS

An expanded, detailed methods section is provided in the supplemental material.

Electrophysiology

The *Xenopus* oocytes expressed with human KCNQ1 and KCNE1 were used to examine the acute effect of amiloride and the dominant negative effect of KCNQ1b on currents (Figure 2). Myocyte whole-cell patch clamp was performed at 36.5°C with Tyrode solution (Figure 3,4). We recorded AP after 6 hours incubation time with or without 50 μ mol/L amiloride.

Myocytes with APDs after stabilization outside the range between 250 ms to 450 ms were not included for analysis.

Biochemistry

To study the effect of amiloride on KCNQ1 splicing, freshly isolated canine myocytes were incubated in either M199 culture medium (Sigma, Saint Louis, MO) or M199 with 50 $\mu\text{mol/L}$ amiloride (Sigma, Saint Louis, MO) at 37 °C in a humidified, 5% CO₂ incubator. The concentration of 50 $\mu\text{mol/L}$ was according to the plasma level in clinical use.¹⁵ After 6 hours, cells were resuspended, washed, and homogenized in Trizol (Invitrogen, Grand Island, NY) for RNA extraction or in protein lysis buffer for protein extraction. Reverse transcriptase-polymerase chain reaction (RT-PCR) of controlled RNA amount (50 ng) was performed by using primers for total KCNQ1, KCNQ1a, and KCNE1. The quantification of mRNA expression levels of total KCNQ1 and KCNQ1a were studied by real-time PCR using a LightCycler instrument (Roche, IN, USA) with the universal probe system. Western blot was performed by specific antibodies shown in Figure 1. Differences were considered statistically significant if $P < 0.05$ (by Student's *t*-test).

Model simulations

The simulations of the cardiac AP are based on a modified O'Hara-Rudy (ORd) model of human ventricular cardiomyocytes, in which the signaling cascade from ISO application to PKA phosphorylation of target proteins was incorporated.^{16–18} The parameters affected by PKA phosphorylation were computed by the Heijman et al model of the β -adrenergic signaling pathway.¹⁷ The I_{Ks} densities used in the simulations during baseline and under ISO for different KCNQ1b/total KCNQ1 ratios were from published data and our experimental results.¹⁰ To determine how the KCNQ1b/total KCNQ1 ratio affects repolarization in the context of heterogeneous heart tissue, simulations on a 1-dimensional transmural wedge were performed.¹⁹

RESULTS

KCNQ1 splicing variants expression was changed by amiloride treatment in canine cardiomyocytes

The effect of amiloride on the KCNQ1 splicing was tested in freshly isolated canine cardiomyocytes. The analysis of RT-PCR products (Fig 1A) indicated that the expression of KCNQ1a was differentially changed by amiloride in Endo, Mid, and Epi (Fig 1B–H). Compared with corresponding controls, the transcript of KCNQ1a was higher in the amiloride-treated group in Mid (1.39 ± 0.08 fold to control) but lower in Endo (0.84 ± 0.06 fold to control) and Epi (0.82 ± 0.02). Transcriptional expression of total KCNQ1 did not differ in Endo, Mid, or Epi between the amiloride-treated and corresponding controls (0.99 ± 0.05 , 0.98 ± 0.04 , and 0.93 ± 0.05 folds to control, respectively). The transcriptional expression of total KCNQ1 and KCNQ1a were also quantified by the real-time PCR (Fig 1L). The expression of total KCNQ1 did not differ in Endo, Mid, or Epi between the amiloride-treated and corresponding controls (1.03 ± 0.04 , 1.08 ± 0.16 , and 1.03 ± 0.07 folds to control, respectively). The transcript of KCNQ1a was higher in the amiloride-treated group in Mid (1.43 ± 0.25 fold to control) but lower in Endo (0.75 ± 0.08 fold to control). Protein expression changes were consistent with transcriptional changes of KCNQ1. The KCNQ1 protein migrates in SDS-PAGE as 75kDa for KCNQ1a, and 60kDa for KCNQ1b (Fig 1G). The densitometry analysis showed that amiloride decreased KCNQ1a and increased KCNQ1b protein expression in Endo (KCNQ1a 0.82 ± 0.04 fold to control, $P < 0.05$; KCNQ1b 1.26 ± 0.10 fold to control, $P < 0.05$) and Epi (KCNQ1a 0.84 ± 0.04 fold to control, $P < 0.05$; KCNQ1b 1.29 ± 0.06 fold to control, $P < 0.05$). Conversely, amiloride increased KCNQ1a and decreased KCNQ1b in Mid (KCNQ1a 1.24 ± 0.06 fold to control, $P < 0.01$;

KCNQ1b 0.85 ± 0.04 fold to control, $P < 0.05$). We also examined the effect of amiloride on the KCNE1 and found that amiloride did not change the transcriptional or translational expression in any of myocyte types (Fig 1B,G,H).

Multiple splicing factors may cause opposite changes in Mid compared Endo and Epi myocytes

Splicing regulation involves a large number of RNA binding proteins, with SR proteins and hnRNPs being the two largest families that function by a wide variety of mechanisms.²⁰ We used Western blot to determine the effect of amiloride on the expression of several splicing associated proteins to examine if the differential regulation of KCNQ1 splicing variants in various types of myocytes by amiloride is a result of the changes of the splicing factors. The splicing factors tested include hnRNP-R, hnRNP-I, hnRNP-C1/C2, hnRNP-A1, SF2, and SRP20 (Fig 1I–K), and the amiloride induced changes of some splicing factors in Mid were opposite to those in Endo and Epi. For instance, with amiloride treatment, hnRNP-R expression significantly increased in Endo and Epi but decreased in Mid myocytes, while hnRNP-C1/C2 and SRP20 expression significantly decreased in Endo and Epi but increased in Mid.

Acute effect of amiloride on KCNQ1 and I_{Ks} channels

The amiloride induced changes in KCNQ1 expression may affect the electrophysiological properties of the heart (see below). To examine if amiloride can also alter heart function by acutely altering I_{Ks} channel function, we treated amiloride on human KCNQ1 and KCNQ1+KCNE1 (I_{Ks}) channels expressed in *Xenopus* oocytes. Amiloride did not change the peak amplitudes of KCNQ1 ($n=4$) or I_{Ks} current ($n=7$) (Fig 2A,B). The G-V relationships of KCNQ1 and I_{Ks} showed that amiloride did not change voltage dependent activation of the channels ($V_{1/2}$ for KCNQ1: amiloride, -22.3 ± 5.2 mV; control, -19.7 ± 6.9 mV, $P > 0.05$; for I_{Ks} : amiloride, 29.7 ± 5.8 mV; control, 30.4 ± 4.9 mV, $P > 0.05$). There is a slope change in the G-V relationship of KCNQ1 after amiloride treatment (amiloride, 14.7 ± 2.4 ; control, 9.5 ± 1.5 , $P = 0.01$). However, the slope for I_{Ks} did not change with amiloride (amiloride, 14.6 ± 2.5 ; control, 13.2 ± 2.6 , $P > 0.05$) (Fig 2C). Amiloride also did not change the activation time (Fig 2D). These experiments demonstrated that amiloride does not directly affect the I_{Ks} channel function.

KCNQ1b exerts dominant negative effect on KCNQ1 current and I_{Ks}

To assess the dominant-negative effect of KCNQ1b quantitatively, we measured the dependence of KCNQ1 and I_{Ks} current amplitude on the KCNQ1b/total KCNQ1 mRNA ratio with a fixed amount of KCNQ1a and KCNE1 mRNA co-injection in *Xenopus* oocytes (Fig 2E,F). The KCNQ1 and I_{Ks} current amplitudes decreased with increasing KCNQ1b/total KCNQ1 mRNA ratio. KCNQ1 and I_{Ks} channels contain four KCNQ1 subunits. It is possible that a channel containing any (1–4) KCNQ1b subunit is not functional. To test this possibility, we assumed that the population of KCNQ1 and I_{Ks} channels containing 0–4 KCNQ1b subunits follows a binomial distribution based on the KCNQ1b/total KCNQ1 mRNA ratio, and only the channels containing no KCNQ1b are functional. This model prediction of the dominant negative effect of KCNQ1b fits well with the experimental results (Fig 2F).

Introduction of the dominant negative effect of KCNQ1b on I_{Ks} into simulations of human ventricular myocyte AP

The amiloride-induced changes of KCNQ1 splicing variants may affect the APs of cardiac myocytes. To examine if amiloride can alter the human cardiac AP, we first did a simulation using Ord model.¹⁶ To define specific I_{Ks} conductance in different types of myocytes for

the AP simulations, we adopted a previous study result showing differential KCNQ1b expression across the human ventricle (Supplemental Table).¹⁰ In their quantitative RT-PCR, KCNQ1b represented $25.2 \pm 2.3\%$, $31.7 \pm 1.2\%$, and $24.9 \pm 1.7\%$ of total KCNQ1 expression in left ventricular Endo, Mid, and Epi tissues, respectively. We used the model prediction of the dominant negative effect of KCNQ1b (Fig 2F) to determine human I_{Ks} conductance based on the KCNQ1b/total KCNQ1 ratio (Fig 3A). Because there is no significant differential expression of total KCNQ1 mRNA (Fig 1C)¹⁰, the KCNQ1b/total KCNQ1 ratios of 0.252 for Endo, 0.317 for Mid, and 0.249 for Epi were used. The normalized I_{Ks} conductance of control human myocytes was thus 0.31 for Endo, 0.22 for Mid, and 0.32 for Epi, respectively (Fig 3A). We then used our biochemistry results (Fig 1) to identify the normalized I_{Ks} conductance upon amiloride treatment. For example, with Endo myocytes, RT-PCR showed KCNQ1a/total ratio of amiloride-treated myocytes is 0.82 fold to control, and Western blot showed a 0.84 fold to control. $\sqrt{0.82 \times 0.84} = 0.83$. Thus, KCNQ1a/total KCNQ1 ratio after amiloride treatment was estimated to be 0.83, 1.31, 0.83 fold to control for Endo, Mid, and Epi myocytes, respectively. Because the total KCNQ1 expression was not significantly different among Endo, Mid, and Epi (Fig 1C), KCNQ1b/total KCNQ1 ratios for amiloride-treated myocytes was 0.380, 0.104, and 0.378 for Endo, Mid, and Epi, respectively. Thereby, the normalized I_{Ks} conductance of human Endo, Mid, and Epi myocytes after amiloride treatment was 0.15, 0.64, and 0.15 (Fig 3A), with a 0.47, 2.96, and 0.47 fold change compared to control, respectively. These factors were used for subsequent AP simulations. For Mid at a CL of 1000 ms simulated APDs were shorter in amiloride treatment (Fig 3B) and the shortening was the same in the absence and the presence of ISO (shortening was 34.4 ms and 29.3 ms; representing 9.2% and 9.9% decrease, respectively). The human AP simulations demonstrated that amiloride-induced KCNQ1b/total KCNQ1 ratio changes in Mid cause APD shortening in the absence and presence of ISO.

APD shortening in amiloride-treated Mid myocytes can be simulated by introducing KCNQ1 splicing related I_{Ks} conductance changes

To examine the above simulation results, we compared AP in Mid of control and amiloride-treated canine myocytes. The myocytes were paced at a CL of 1000 ms (Fig 3C,D). The APD (mean $\pm$ S.D.) was significantly shortened by amiloride treatment in the absence of ISO, from 368 ± 38 ms in control to 330 ± 33 ms in amiloride-treated Mid under baseline condition ($P=0.01$) and the shortening was from 289 ± 39 ms in control to 249 ± 31 ms in amiloride-treated with ISO ($P=0.16$) (Fig 3C,D).

In addition, the difference of APD between control and amiloride-treated Mid increased while the pacing CL shortened (faster rate) in both experiments and model simulations. Experimentally, APD in amiloride treated myocytes was 11.7%, 10.4%, and 7.5% shorter than in control at CLs of 500, 1000, and 2000 ms, respectively. In human Mid myocyte AP simulations, the APD was shortened by increasing I_{Ks} conductance 2.96 times (Fig 3A), and the APD shortening was also enhanced at fast rates (9.7%, 9.2%, and 8.8% shorter than in control at CLs of 500, 1000, and 2000 ms, respectively) (Fig 3E). This phenomenon is due to an important I_{Ks} role in rate adaptation of APD, by which the I_{Ks} facilitates AP repolarization acceleration and APD shortening at fast rates.³ The frequency-dependence of amiloride induced APD shortening in both experiments and simulation further supports that the APD shortening was derived from enhanced I_{Ks} current by amiloride treatment.

Amiloride treatment reduced occurrence of EADs with ISO challenge in Mid

For canine left ventricular myocytes, ISO at concentrations as low as 20 nmol/L can induce EADs.²¹ To examine if amiloride can affect the development of ISO-induced EADs at both slow and fast rate, we performed AP recordings in canine Mid with ISO challenge by

gradual titration. The threshold of ISO concentration for inducing EADs in our canine Mid was 20 or 50 nmol/L and was not different between control and amiloride-treated myocytes. However, EADs were generated more frequently in control than in amiloride-treated myocytes (Fig 4A–C). In both control and amiloride-treated Mid, EADs were more frequently generated at slower rates (Fig 4C).

To evaluate the impact of amiloride on EAD generation, we used the ORd model in which a series of modifications on the equations to account for β -adrenergic stimulation were incorporated (see supplemental material). Previous studies had shown that under steady state pacing at 1000 ms CL, the application of 1 μ mol/L ISO led to EAD generation even in normal Mid AP simulations, partially due to the fact that ISO-induced phosphorylation is faster in I_{CaL} than in I_{Ks} .^{18,22} Our simulations revealed that at slow pacing EADs appeared in both control and amiloride-treated Mid after ISO application (Fig 4D). Less EADs were generated at fast rate, and they were inhibited by amiloride. The EADs were associated with I_{CaL} reactivation, while the increased I_{Ks} conductance caused shortening of APD and reduced I_{CaL} reactivation. EAD was not noted in either Endo or Epi AP simulations under ISO challenge (data not shown). The simulations suggest that amiloride induced 2.96-fold I_{Ks} conductance increase can inhibit EADs generation in human ventricular Mid under the challenge of high concentrations of ISO.

DISCUSSION

The results of the present study suggest that (1) amiloride changes the alternative splicing of KCNQ1 and that effect may be caused by splicing factors changes; (2) a prominent I_{Ks} current density modulation can be attributed to a small change in KCNQ1b splicing variant expression; and (3) in Mid myocytes, increased I_{Ks} density shortens APD and inhibits EADs development under beta-adrenergic stimulation.

Amiloride has multiple pharmacological actions on ionic channels and transporters. It inhibits the Na/H exchanger in cultured cardiac cells and in sheep Purkinje fibers, nonetheless Na/H exchange inhibition by 0.01 to 1.0 mmol/L amiloride does not cause intracellular acidosis.²³ Amiloride also suppresses the T-type calcium current in guinea pig ventricular myocytes with a Kd of 233 μ mol/L and inhibits sarcolemmal Na^+/Ca^{2+} exchanger with EC₅₀ at 0.7 mmol/L in isolated beating guinea pig atria.^{24,25} The concentration of 50 μ mol/L used in this study is too low to affect the intracellular pH or to inhibit T-type calcium current and the Na^+/Ca^{2+} exchanger. Amiloride of 10 μ mol/L was shown to prolong the APD with prolonged treatment in canine cardiac Purkinje fibres.²⁶ If amiloride could change KCNQ1 splicing (with increased KCNQ1b expression) in Purkinje fibers remains to be determined.

Other genetic factors have been shown to modify KCNQ1 transcript expression and QT interval, e.g. variants in the 3' untranslated region of KCNQ1, variants in putative ion channel regulatory subunits such as NOS1AP, and stimulating protein 1 which is involved in transcriptional activation of KCNQ1 promoter.^{27–29} To our best knowledge, this is the first report describing drug-induced alternative splicing of KCNQ1 in cardiac myocytes. The detailed mechanism involved in KCNQ1 splicing remains to be investigated.

Clinical implications

QT dispersion, which is the difference of minimal QT interval and maximal QT interval in the surface 12-lead electrocardiogram (ECG), has been suggested by some to reflect repolarization inhomogeneity in the heart and to have association with sudden cardiac death in healthy subjects and in patients with chronic heart failure.^{30,31} In a double-blinded clinical study, amiloride significantly reduced QT dispersion and also reduced ventricular

extrasystoles.³² Simulated pseudo-ECG establishes a direct relationship between ionic currents, the AP, and the morphology of electrocardiographic waveforms. It allows us to evaluate the impact of amiloride on the ECG QT interval of ECG without performing an in vivo study (Fig 5). We introduced the estimated I_{Ks} conductance of Endo, Mid, and Epi in the amiloride simulations as 0.47, 2.96, and 0.47 times control, respectively (Fig 3A, 5). Moreover, due to the nature of potassium sparing diuretics, amiloride increases plasma potassium by an average value of 0.5 mmol/L.^{13,14,32} Therefore, we further simulated APs and pseudo-ECGs with increased extracellular K^+ level by 0.5 mmol/L (Fig 5). In AP simulations, the reduced I_{Ks} conductance caused APD prolongation with 8 ms for Endo and 4.7 ms for Epi (Fig 5); however, these prolongations were offset by the elevation of K^+ level (APD changes were only 0.8 ms for Endo and -0.9 ms for Epi). The above clinical study also observed that the potassium elevation shortened QT interval.³² The heterogeneity of the I_{Ks} conductance among three transmural layers was enlarged by amiloride (Fig 3A), nonetheless APD differences and TDR were reduced because of major increase of Mid I_{Ks} and shortening of its APD (Fig 5). The reduction of TDR by amiloride was frequency-dependent and was larger at a faster pacing rate (14%, 16.7%, and 25.8% reduction with amiloride comparing to the control, at CLs of 2000, 1000, and 500 ms, respectively) (Fig S1). These results suggest that reduced dispersion may be an antiarrhythmic mechanism of amiloride in the clinical setting.³¹ This study also suggests that alternative splicing of KCNQ1 may be an anti-arrhythmic therapeutic target.

In conclusion, amiloride induces KCNQ1 splicing changes through affecting expression of splicing factors, and the effects show transmural differences. This drug-induced modulation of I_{Ks} due to alternative splicing of KCNQ1 changes the I_{Ks} transmural heterogeneity, results in reduced transmural dispersion, reduced EAD formation and therefore reduced arrhythmogeneity under α -adrenergic stimulation. These changes are suggested to contribute to a decreased arrhythmogeneity as a beneficial effect of amiloride.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AP	action potential
APD	action potential duration
CL	cycle length
EAD	early afterdepolarization
Endo	endocardial myocyte

Epi	epicardial myocyte
I_{CaL}	L-type calcium current
I_{Kr}	rapid delayed rectifier potassium current
I_{Ks}	slow delayed rectifier potassium current
ISO	isoproterenol
Mid	midmyocardial myocyte
TDR	transmural dispersion of repolarization

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FIGURE 1. Amiloride treatment alters splicing variants of KCNQ1 and splicing factors in canine cardiomyocytes

A. Primers for the reverse-transcriptase PCR (RT-PCR) of KCNQ1 and the PCR products reflect the transcriptional amounts of KCNQ1a (362bp) and total KCNQ1 (160bp). **B.** The representative RT-PCR products electrophoresis of KCNQ1a, total KCNQ1, KCNE1 and GAPDH in Endo, Mid, and Epi. **C&D.** The relative transcriptional expression levels of total KCNQ1 and of KCNQ1a vs. GAPDH (n=4 for each group in RT-PCR). **E.** Expression under amiloride compared with corresponding controls. **F.** Transcriptional expression of KCNE1 of control and amiloride-treated groups. **G.** KCNQ1 and KCNE1 protein expression shown by Western blot; 75kDa for KCNQ1a, and 60kDa for KCNQ1b. Three bands of KCNE1

represent double-glycosylated (30kDa), single-glycosylated (20kDa), and un-glycosylated KCNE1 protein (16kDa) respectively (n=6 for each group). **H.** Densitometry analysis of western blots for the effect of amiloride on KCNQ1a, KCNQ1b, and KCNE1. **I.** Western blots how splicing factors hnRNP-R, hnRNP-C1/C2, hnRNP-A2/B1, and SRP20 of control (C) and amiloride-treated (A) groups. **J.** Densitometry analysis of western blots for the effect of amiloride on splicing factors. **K.** Coomassie blue of the same SDS-PAGE gel as in **I.** **L.** Real-time PCR for transcriptional expression of total KCNQ1 and KCNQ1a under amiloride treatment (n=3 for each group).

FIGURE 2. Acute effect of amiloride and the effect of KCNQ1b on KCNQ1 and I_{Ks} currents

A. Human KCNQ1 and I_{Ks} (KCNQ1a+KCNE1) currents expressed in *Xenopus* oocytes before and after acute amiloride treatment. Currents were elicited by voltage pulses from -80 mV to $+60$ mV with 10 mV increment; the holding and repolarization potentials were -80 and -40 mV, respectively. **B.** KCNQ1a and I_{Ks} peak tail currents following depolarization to $+40$ mV at various time ($n=3$ for KCNQ1a and $n=6$ for I_{Ks}). Open symbol is for currents without amiloride application during the entire recordings (control); filled symbol is for currents recorded before and during amiloride application with the gray box indicating the period of administration (amiloride). **C.** G-V relations of normalized KCNQ1a and I_{Ks} tail currents with and without amiloride treatment ($n=5$ for each). **D.** Voltage dependence of activation times of KCNQ1a and I_{Ks} currents. The τ_{As} and τ_{Af} are obtained from fitting the activating current traces with a double exponential function. **E.** I_{Ks} currents from oocytes with injection of 4 ng KCNQ1a, 3.5 ng KCNE1, and 0 – 8 ng KCNQ1b mRNA. **F.** I_{Ks} and KCNQ1 current amplitudes depend on the KCNQ1b/total KCNQ1 mRNA ratio. Peak currents were normalized to that without KCNQ1b co-injection. The curve of predicted dominant negative effect (see text) is in dotted line.

FIGURE 3. APD shortening in amiloride-treated Mid myocytes can be simulated by introducing KCNQ1 splicing related I_{Ks} conductance changes

A. Simulation of normalized I_{Ks} current densities based on the KCNQ1b/total KCNQ1 ratio (see text). Arrows indicate I_{Ks} current densities. **B.** Simulations of APs and corresponding I_{Ks} current densities in control and amiloride-treated Endo, Mid, and Epi using a human ventricular model.¹⁶ **C.** AP recordings of control and amiloride-treated canine Mid in the baseline and after isoproterenol (ISO) (10 nmol/L) administration. CL: pacing cycle length. **D.** APD measurement at pacing CL of 1000 ms in canine Mid in the absence and presence of ISO. **E.** Frequency-dependence of APD shortening recorded from canine Mid and model

simulation of human Mid. Increased I_{Ks} conductance simulates the effect of amiloride effects.

FIGURE 4. Amiloride treatment reduced isoproterenol (ISO)-induced EAD in isolated Mid
A. Representative traces of continuous AP recordings from canine Mid with and without amiloride treatment. Baseline are shown in gray, with ISO (20 or 50 nmol/L) are in black. **B.** APs at pacing cycle lengths (CL) of 2000, 1000, and 500 ms with and without amiloride treatment. Baseline APs are in grey and with ISO are in black. **C.** The mean number of EAD ($\pm$ SEM) per 10 consecutive beats during steady-state pacing. * indicates statistically significant ($P < 0.05$). The number of cells recorded, $n = 3, 4, 2$ for control and $n = 3, 3, 2$ for amiloride treated, respectively. **D.** Simulations of ISO-induced EADs in human Mid. Descending rows show the AP, I_{CaL} , and I_{Ks} . Columns, from left to right, show simulations

at CLs of 2000 ms, 1000 ms, and 500 ms under ISO. Gray lines illustrate controls. Black lines illustrate simulations of amiloride effect.

FIGURE 5. Amiloride reduces transmural dispersion of repolarization (TDR) due to the I_{Ks} conductance changes

Simulated APs of (top to bottom) human endocardial (Endo), midmyocardial (Mid), and epicardial (Epi) myocytes, together with a simulated pseudo-ECG (ECG). Columns, from left to right, show cases of control, amiloride effect with I_{Ks} conductance changes, and amiloride with both I_{Ks} conductance change and potassium (K^+) elevation. The QT interval and TDR are indicated.