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

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BACKGROUND Slow delayed-rectifier potassium current (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 To study the modulation of KCNQ1 alternative splicing by amiloride and the consequent changes in I_{Ks} and action potentials (APs) 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 using the reverse transcriptase-polymerase chain reaction and Western blot. The effect of amiloride-induced changes in the KCNQ1b/total KCNQ1 ratio on AP was measured by using whole-cell patch clamp with and without isoproterenol.

RESULTS With 50 μmol/L of amiloride for 6 hours, KCNQ1a at transcriptional and translational levels increased in midmyocardial myocytes but decreased in endo- and epicardial myocytes. Likewise, changes in 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 AP simulations under beta-adrenergic stimulation. Moreover, amiloride reduced the transmural dispersion of repolarization in pseudo-electrocardiogram.

CONCLUSIONS Amiloride regulates I_{Ks} and APs with transmural differences and reduces arrhythmogenicity through the modulation of KCNQ1 splicing. We suggested that the modulation of KCNQ1 splicing may help prevent arrhythmia.

KEYWORDS Action potential; Alternative splicing; Amiloride; I_{Ks}; KCNQ1

ABBREVIATIONS **AP** = action potential; **APD** = action potential duration; **CL** = cycle length; **EAD** = early afterdepolarization; **ECG** = electrocardiogram; **Endo** = endocardial; **Epi** = epicardial; **I_{CaL}** = L-type calcium current; **I_{Ks}** = slow delayed-rectifier potassium current; **ISO** = isoproterenol; **Mid** = midmyocardial; **ORd** = O'Hara-Rudy; **PKA** = protein kinase A; **RT-PCR** = reverse transcriptase-polymerase chain reaction; **TDR** = transmural dispersion of repolarization

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Introduction

Ventricular repolarization is significantly dependent on slow delayed-rectifier potassium current (I_{Ks}), and the contribution of I_{Ks} to action potential (AP) repolarization is enhanced during beta-adrenergic stimulation.[1–4] This enhancement is also reflected in 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 protein kinase A (PKA) following beta-adrenergic receptor stimulation greatly enhances I_{Ks} by increasing current amplitude, by leftward shift in the current-voltage 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

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repolarization currents, in particular rapid delayed-rectifier potassium current.[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 than Mid myocytes.[5,6] In the human and the canine heart, there are 2 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 owing to a trafficking defect, and KCNQ1b transgenic mice manifest with pronounced QT prolongation and ventricular arrhythmias.[7–9] 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.[9] However, whether splicing of KCNQ1 can be altered and how such alterations affect 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.[10,11] Prolonged treatment with amiloride also exerts an antiarrhythmic effect both in postinfarction dogs and in patients with ventricular tachycardia.[12,13] However, 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} densities, as well as investigate the effect of KCNQ1 splicing on the action potential duration (APD), isoproterenol (ISO)-induced EAD, and cardiac transmural dispersion of repolarization (TDR). The mechanism of how amiloride affects arrhythmogenicity is also addressed.

Methods

An expanded, detailed methods section is provided in the Online 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 (see Figure 2). Myocyte whole-cell patch clamp was performed at 36.5°C with Tyrode's solution (see Figures 3 and 4). We recorded AP after 6 hours of incubation time with or without 50 μmol/L of amiloride. Myocytes with APDs after stabilization outside the 250–450 ms range 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, St Louis, MO) or M199 with 50 μmol/L of amiloride (Sigma) at 37°C in a humidified 5% CO_2 incubator. The amiloride concentration of 50 μmol/L was according to the plasma level in clinical use.[14] 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. The reverse transcriptase-polymerase chain reaction (RT-PCR) of the 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 performing real-time PCR using a LightCycler instrument (Roche, Indianapolis, IN) with the universal probe system. Western blotting was performed by using specific antibodies shown in Figure 1. Differences were considered statistically significant if $P < .05$ (by the Student t test).

Model simulations

The simulations of cardiac APs 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.[15–17] The parameters affected by PKA phosphorylation were computed by using the Heijman et al model of the beta-adrenergic signaling pathway.[16] 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.[9] 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.[18]

Results

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

The effect of amiloride on KCNQ1 splicing was tested in freshly isolated canine cardiomyocytes. The analysis of RT-PCR products (Figure 1A) indicated that the expression of KCNQ1a was differentially changed by amiloride in Endo, Mid, and Epi myocytes (Figures 1B–1H). Compared to the corresponding control myocytes the transcript of KCNQ1a was higher in the amiloride-treated group in Mid myocytes (1.39 ± 0.08 fold to control) but lower in Endo (0.84 ± 0.06 fold to control) and Epi (0.82 ± 0.02 fold to control) myocytes. The transcriptional expression of total KCNQ1 did not differ in Endo, Mid, or Epi myocytes between the amiloride-treated and the corresponding control group (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 (Figure 1L). The expression of total KCNQ1 did not differ in Endo, Mid, or Epi myocytes between the amiloride-treated and corresponding control groups (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 myocytes (1.43 ± 0.25 fold to control) but lower in Endo myocytes (0.75 ± 0.08 fold to control). Protein