Journal of Molecular and Cellular Cardiology 47 (2009) 743–747

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Rapid communication

Action potential clamp and chloroquine sensitivity of mutant Kir2.1 channels responsible for variant 3 short QT syndrome

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ARTICLE INFO

Article history:
Received 15 October 2008
Received in revised form 10 February 2009
Accepted 19 February 2009
Available online 10 March 2009

Keywords:
Arrhythmia
Antiarrhythmic
Chloroquine
KCNJ2
Kir2.1
QT interval
Short QT syndrome
Sudden death

ABSTRACT

Recently identified genetic forms of short QT syndrome (SQTS) are associated with an increased risk of arrhythmia and sudden death. The SQT3 variant is associated with an amino-acid substitution (D172N) in the *KCNJ2*-encoded Kir2.1 K^+ channel. In this study, whole-cell action potential (AP) clamp recording from transiently transfected Chinese Hamster Ovary cells at 37 °C showed marked augmentation of outward Kir2.1 current through D172N channels, associated with right-ward voltage-shifts of peak repolarizing current during both ventricular and atrial AP commands. Peak outward current elicited by ventricular AP commands was inhibited by chloroquine with an IC_{50} of 2.45 μM for wild-type (WT) Kir2.1, of 3.30 μM for D172N-Kir2.1 alone and of 3.11 μM for co-expressed WT and D172N ($P>0.05$ for all). These findings establish chloroquine as an effective inhibitor of SQT3 mutant Kir2.1 channels.

1. Introduction

The short QT syndrome (SQTS) is linked to abbreviated QT intervals on the electrocardiogram and to an increased incidence of cardiac arrhythmias and of sudden death [1]. The SQT3 variant was identified in an asymptomatic child with an abnormal electrocardiogram and in her father who had a history of presyncopal events and palpitations [2]. Father and daughter had abbreviated rate-corrected QT (QT_c) intervals of 320 ms and 315 ms respectively, and programmed electrical stimulation was able to elicit ventricular fibrillation [2]. Genetic analysis [2] revealed that neither individual exhibited mutations in *KCNH2* or *KCNQ1* (associated with SQT1 and SQT2 variants respectively; [1]), but a single base substitution was identified in *KCNJ2*, giving rise to an aspartate to asparagine substitution at position 172 (D172N) in the Kir2.1 potassium channel protein. In humans, Kir2.1 is expressed in both atria and ventricles and contributes to channels underlying the inwardly rectifying K^+ current I_{K1} [3]. Patch clamp recordings at ambient temperature showed augmentation of outward but not inward current through D172N-Kir2.1 channels, predicted in cell and tissue simulations to accelerate ventricular repolarization [2].

For the *KCNH2*-linked SQT1 variant, the action potential voltage-clamp ('AP clamp') technique has been used to characterize effects of the SQT1 mutation on ionic current during physiological waveforms [4,5]. In addition, *in vitro* and *in vivo* investigations have also identified pharmacological agents that can help restore towards normal the QT intervals of SQT1 patients (e.g. [6–8]). To date, neither of these approaches has been applied to the SQT3 D172N mutation. Accordingly, the present study: (i) provides the first AP clamp information on effects of the Kir2.1 mutation, and does so at physiological temperature; (ii) identifies an effective pharmacological inhibitor of SQT3 D172N mutant Kir2.1.

2. Methods

2.1. Maintenance of cells expressing WT and D172N-Kir2.1 channels

Wild-type (WT) and mutant (D172N) Kir2.1 (in pSVL expression vector) were kindly provided by Professor H Matsuda [9]. Chinese Hamster Ovary (CHO) cells were passaged using a non-enzymatic agent (Enzyme Free, Chemicon®International) and then maintained as described previously [5]. They were transiently transfected with either WT or D172N-Kir2.1 (2 μg of each construct was used) at a ratio of 4:1 with CD8 (in pIRES; courtesy of Dr I Baró and Dr J Barhanin), 24 h after plating cells out, using Lipofectamine™ LTX (Invitrogen), according to the manufacturer's instructions. For co-expression of WT and D172N-Kir2.1 (to mimic the heterozygous condition of the SQT3 proband [2]) cells were transfected with equal amounts of WT and

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doi:10.1016/j.yjmcc.2009.02.027

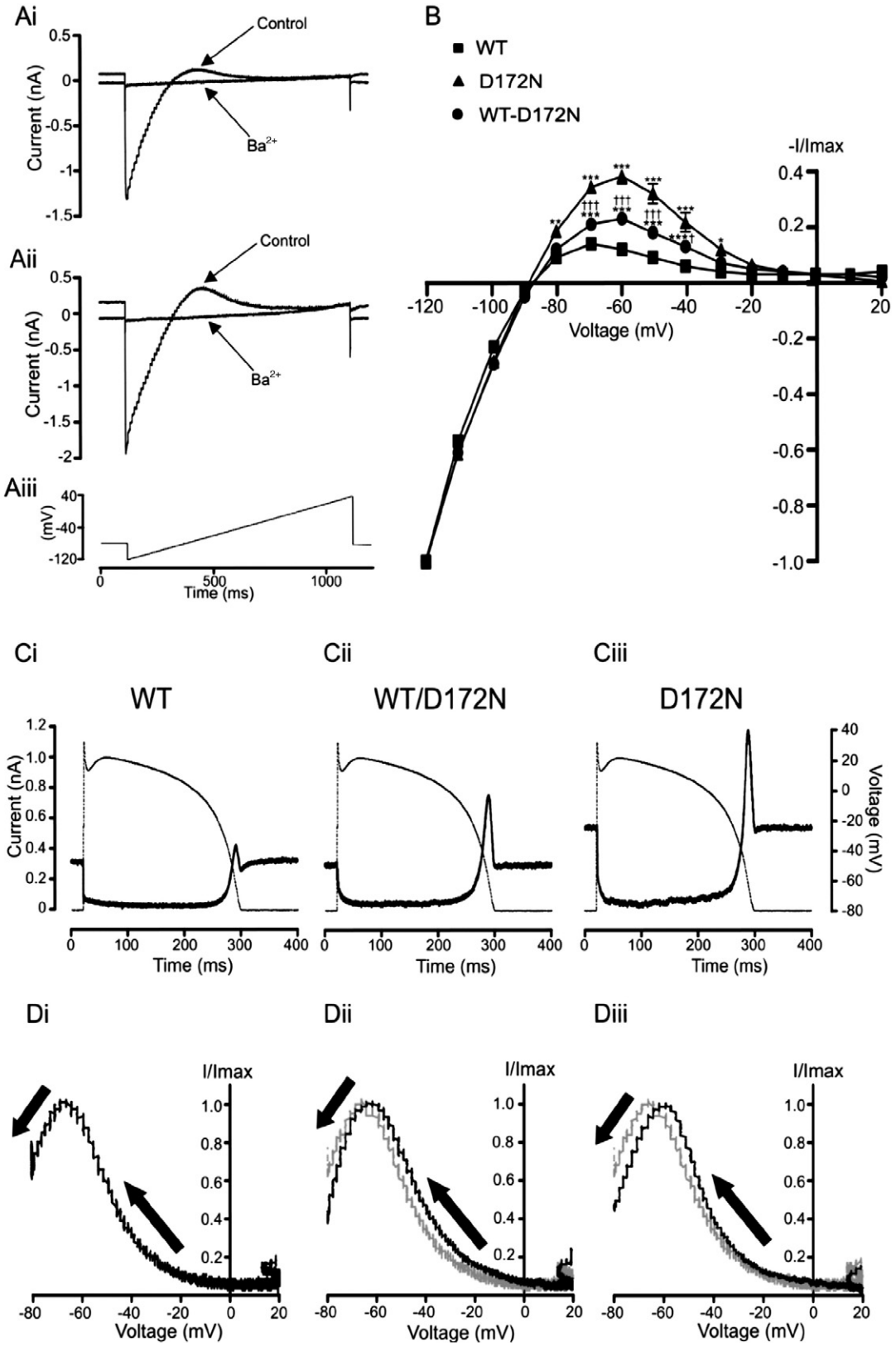

Fig. 1. WT, WT-D172N and D172N-Kir2.1 during ramp and ventricular AP waveforms. (A) Example traces of WT $I_{Kir2.1}$ (Ai) and D172N $I_{Kir2.1}$ (Aii) elicited by ascending voltage ramp command, shown in (Aiii) (applied at 3 s intervals) in control and following application of 1 mM $BaCl_2$. (B) Mean current–voltage (*I–V*) relations for Ba^{2+}-sensitive current for WT, WT-D172N and D172N Kir2.1 ($n = 18$, 24 and 7 cells respectively). For each cell, currents were normalized to the current at −120 mV to facilitate comparison between the three channel expression conditions. (***$P<0.001$ versus WT, **$P<0.01$ versus WT,*$P<0.05$ versus WT, †††$P<0.001$ versus D172N, †$P<0.05$ versus D172N). (C) Profile of 1 mM Ba^{2+}-sensitive WT $I_{Kir2.1}$ (Ci), WT-D172N $I_{Kir2.1}$ (Cii) and D172N $I_{Kir2.1}$ (Ciii) (solid traces) during an epicardial ventricular AP command (dash-dotted trace, 1 Hz). Residual capacitative current transients during the rising phase of the AP command have been blanked for clarity of display. (D) Representative instantaneous *I–V* relations for WT (Di), WT-D172N (Dii) and D172N (Diii) current during ventricular AP repolarization (direction of repolarization denoted by arrows). For each cell, currents were normalized to the maximal current during repolarization and plotted against the corresponding membrane potential from the AP peak to the return to −80 mV. To facilitate comparison between the different channel expression conditions the WT *I–V* relation was superimposed (as a grey trace) on the WT-D172N (Dii) and D172N (Diii) *I–V* plots (black traces).