Cardiac-specific ablation of synapse-associated protein SAP97 in mice decreases potassium currents but not sodium current

Ludovic Gillet, PhD,[*] Jean-Sébastien Rougier, PhD,[*] Diana Shy, MSc,[*] Stephan Sonntag, PhD,[†] Nathalie Mougenot, PhD,[‡] Maria Essers, BSc,[*] Doron Shmerling, PhD,[†] Elise Balse, PhD,[§¶‖] Stéphane N. Hatem, MD, PhD,[§¶‖] Hugues Abriel, MD, PhD[*]

From the [*]Department of Clinical Research, University of Bern, Bern, Switzerland, [†]PolyGene AG, Rümlang, Switzerland, [‡]Plateau d'Expérimentation Coeur, Muscle, Vaisseaux, Université Pierre et Marie Curie, Paris, France, [§]Institut National de la Santé et de la Recherche Médicale (INSERM), UMR S1166, Institut de Recherche Sur Les Maladies Cardiovasculaires, du Métabolisme et de la Nutrition, Paris, France, [¶]Sorbonne Universités, UPMC Univ Paris 06, UMR S1166, Paris, France, and [‖]Institute of Cardiometabolism & Nutrition, ICAN, Pitié-Salpêtrière Hospital, Paris, France.

BACKGROUND Membrane-associated guanylate kinase (MAGUK) proteins are important determinants of ion channel organization in the plasma membrane. In the heart, the MAGUK protein SAP97, encoded by the *DLG1* gene, interacts with several ion channels via their PDZ domain-binding motif and regulates their function and localization.

OBJECTIVE The purpose of this study was to assess *in vivo* the role of SAP97 in the heart by generating a genetically modified mouse model in which SAP97 is suppressed exclusively in cardiomyocytes.

METHODS SAP97$^{fl/fl}$ mice were generated by inserting loxP sequences flanking exons 1–3 of the SAP97 gene. SAP97$^{fl/fl}$ mice were crossed with αMHC-Cre mice to generate αMHC-Cre/SAP97$^{fl/fl}$ mice, thus resulting in a cardiomyocyte-specific deletion of SAP97. Quantitative reverse transcriptase–polymerase chain reaction, western blots, and immunostaining were performed to measure mRNA and protein expression levels, and ion channel localization. The patch-clamp technique was used to record ion currents and action potentials. Echocardiography and surface ECGs were performed on anesthetized mice.

RESULTS Action potential duration was greatly prolonged in αMHC-Cre/SAP97$^{fl/fl}$ cardiomyocytes compared to SAP97$^{fl/fl}$ controls, but maximal upstroke velocity was unchanged. This was consistent with the decreases observed in I_{K1}, I_{to}, and I_{Kur} potassium currents and the absence of effect on the sodium current I_{Na}. Surface ECG revealed an increased corrected QT interval in αMHC-Cre/SAP97$^{fl/fl}$ mice.

CONCLUSION These data suggest that ablation of SAP97 in the mouse heart mainly alters potassium channel function. Based on the important role of SAP97 in regulating the QT interval, *DLG1* may be a susceptibility gene to be investigated in patients with congenital long QT syndrome.

KEYWORDS SAP97; Sodium channel; Potassium channel; Action potential; Tissue-specific knockout

ABBREVIATIONS **AP** = action potential; **APD** = action potential duration; **ECG** = electrocardiogram; **I_{K1}** = inward rectifier potassium current; **I_{Kur}** = ultrarapid delayed rectifier potassium current; **I_{Na}** = sodium current; **I_{ss}** = sustained potassium current; **I_{to}** = transient outward potassium current; **QTc** = corrected QT interval; **RT-PCR** = reverse transcriptase–polymerase chain reaction; **SAP97** = synapse-associated protein-97

(Heart Rhythm 2015;12:181–192) © 2015 Heart Rhythm Society. All rights reserved.

Introduction

Proteins of the membrane-associated guanylate kinase (MAGUK) family are characterized by numerous protein–protein interaction domains, including the PDZ domains.[1] PDZ is an acronym combining the first letters of 3 proteins: postsynaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 (ZO-1), which were first discovered to share this domain.[2] The PDZ domains have common structures with 80–90 amino acids.

The research leading to these study results received funding from the European Community's Seventh Framework Program FP7/2007–2013 under grant agreement No. HEALTH-F2-2009-241526, from EUTrigTreat (to Drs. Abriel and Shmerling), and from the Swiss National Science Foundation to Dr. Abriel (310030B_14706035693). We are grateful to the Berne University Research Foundation. **Address reprint requests and correspondence:** Dr. Ludovic Gillet, University of Bern, Department of Clinical Research, Murtenstrasse 35, 3010 Bern, Switzerland. E-mail address: ludovic.gillet@dkf.unibe.ch. or Dr. Hugues Abriel, University of Bern, Department of Clinical Research, Murtenstrasse 35, 3010 Bern, Switzerland. E-mail address: hugues.abriel@dkf.unibe.ch.

1547-5271/$-see front matter © 2015 Heart Rhythm Society. All rights reserved. http://dx.doi.org/10.1016/j.hrthm.2014.09.057

MAGUKs regulate the function and localization of many ion channels in neurons and epithelial cells, mostly at cell-to-cell junctions.[1] However, little is known about their function in the heart.[3] SAP97 (synapse-associated protein-97) and ZO-1 (zonula occludens-1) are the main MAGUK proteins expressed in cardiomyocytes.[3]

SAP97 (also called Dlg1 or Dlgh1, for Disks large homolog 1, encoded by the *DLG1* gene) is involved in the targeting of receptor and channel proteins to specialized domains of the plasma membranes and in the modulation of ion channel activity.[1,4] SAP97 is expressed in cardiomyocytes,[3,5] in which it has been shown to associate with and regulate several ion channels. Leonoudakis et al[6] first identified a direct association of the inwardly rectifying potassium channel $K_{ir}2.2$ with SAP97 in rat cardiac ventricular myocytes. Using a truncated form of the C-terminal GST-$K_{ir}2.2$ fusion protein, they showed that the last 3 amino acids (SEI) were essential for association with SAP97.[6] Using affinity pull-down experiments, they also demonstrated that each of the $K_{ir}2.1$, $K_{ir}2.2$, $K_{ir}2.3$, and $K_{ir}4.1$ channels can interact with distinct scaffolding proteins (dystrophin-associated complex, SAP97, CASK, Veli), and that there is channel specificity in the interaction.[7] Subsequently, Vaidyanathan et al[8] reported that silencing SAP97 in adult rat ventricular myocytes reduces the whole-cell density of the inward rectifier potassium current I_{K1} by reducing the number of $K_{ir}2.x$ channels. They also presented evidence that silencing SAP97 blunted the β_1-adrenergic receptor-mediated regulation of I_{K1}, suggesting that this scaffolding protein is important for assembling a macromolecular signaling complex. Other cardiac potassium channels have also been shown to interact with SAP97. Godreau et al[3] reported that SAP97 is abundantly expressed in human atrial myocardium and that immunoprecipitation of SAP97 co-precipitated $K_V1.5$ channels and *vice versa*. It was also shown that adenovirus-mediated SAP97 overexpression in rat cardiac myocytes resulted in the clustering of endogenous $K_V1.5$ subunits at myocyte–myocyte contacts and in an increase in both the maintained component of the outward potassium current I_{Kur} and the number of 4-aminopyridine-sensitive potassium channels in cell-attached membrane patches.[9] In another study using rat ventricular or human atrial lysates for pull-down experiments with GST fusion proteins comprising the last 50 residues of $K_V4.2$ and $K_V4.3$ channels, our groups found that SAP97 also interacts with these channels, which account for a large part of the outward potassium current I_{to} in the heart.[10] These findings suggest that SAP97 and cardiac $K_V4.x$ channel subunits interact directly via the C-terminus of the channels. Moreover, it was observed that SAP97 and $K_V4.3$ channels co-localize at the plasma membrane of rat atrial myocytes and that SAP97 modulates cardiac I_{to}.

In a recent study, our groups demonstrated a specific interaction between the PDZ domain-binding motif of $Na_V1.5$ (SIV) and SAP97 in mouse ventricles and human atria.[11] Immunostainings performed on rat heart sections showed that both $Na_V1.5$ and SAP97 are localized at intercalated discs, and electrophysiologic studies demonstrated that the sodium current I_{Na} was reduced in rat atrial myocytes that were infected with SAP97 shRNA-containing lentiviruses. More recently, Milstein et al[12] found that $Na_V1.5$, $K_{ir}2.1$, and SAP97 are parts of the same macromolecular complex. Moreover, they found a reciprocal modulation between $Na_V1.5$ and $K_{ir}2.1$.

Although different experimental models have been used to study cardiac ion channel regulation by SAP97, thus far no data are available regarding the role of SAP97 in regulating heart function *in vivo*. Two total knockout SAP97 mouse models have been previously generated (13,14), and in both cases, severe organ malformations led to early death right after birth.[13,14] No cardiac malformation was reported.

Because ion channel interacting proteins are considered important actors in ion channel physiology and pathophysiology,[15] we investigated the *in vivo* significance of SAP97 in the mouse heart by generating a genetically modified mouse model in which SAP97 expression was constitutively suppressed in cardiomyocytes, and we studied the consequences on cardiac ion channel function and heart activity.

Methods and materials

All experiments involving animals were performed according to the Swiss Federal Animal Protection Law and were approved by the Cantonal Veterinary Administration, Bern. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Generation of conditional SAP97 knockout mice

A conditional mouse model was generated for SAP97/Dlg1, which allows tissue or time-point specific inactivation of the Dlg1 gene by use of the Cre-/loxP-system (Figure 1; see Online Supplementary Data for a detailed description).

RNA extraction and quantitative reverse transcriptase–polymerase chain reaction

RNA extraction and quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) procedures are described in detail in the Online Supplementary Data.

Protein extraction and western blot

These procedures were performed as previously reported (see Online Supplementary Data for a detailed description).[16]

Immunohistochemistry of mouse ventricular sections

Immunostainings were performed on heart cryosections as previously described.[16]

Download English Version:

https://daneshyari.com/en/article/5959983

Download Persian Version:

https://daneshyari.com/article/5959983

Daneshyari.com