Journal of Molecular and Cellular Cardiology 46 (2009) 644–652

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

Normal impulse propagation in the atrioventricular conduction system of Cx30.2/Cx40 double deficient mice

Jan W. Schrickel [a,*,1], Maria M. Kreuzberg [b,1], Alexander Ghanem [a], Jung-Sun Kim [c], Markus Linhart [a], Rene Andrié [a], Klaus Tiemann [a], Georg Nickenig [a], Thorsten Lewalter [a,2], Klaus Willecke [b,2]

[a] Department of Medicine Cardiology, University of Bonn, Sigmund-Freud Strasse 25, D-53105 Bonn, Germany
[b] Institute of Genetics, Division of Molecular Genetics, University of Bonn, Römerstr. 164, 53117 Bonn, Germany
[c] Department of Pathology, University of Ulsan, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 18 June 2008
Received in revised form 16 January 2009
Accepted 7 February 2009
Available online 24 February 2009

Keywords:
AV-node
Cardiac conduction
Gap junctions
Electrical coupling
Connexin40
Connexin30.2
Connexin45
Atrial fibrillation
Cx30.2/Cx40 double deficient mouse

ABSTRACT

Connexin (Cx) 30.2, Cx40 and Cx45 containing gap junctional channels contribute to electrical impulse propagation through the mouse atrioventricular node (AV-node). The cross talk in between these Cxs may be of great importance for AV-nodal conduction. We generated Cx30.2/Cx40 double deficient mice ($Cx30.2^{LacZ/LacZ}Cx40^{-/-}$) and analyzed the relative impact of Cx30.2 and Cx40 on cardiac conductive properties in vivo by use of electrophysiological examination. $Cx30.2^{LacZ/LacZ}Cx40^{-/-}$ mice exhibited neither obvious cardiac malformations nor impaired contractile function. In surface-ECG analyses, $Cx30.2^{LacZ/LacZ}Cx40^{-/-}$ and Cx40 deficient animals ($Cx40^{-/-}$) showed significantly longer P-wave durations, PQ-intervals and prolonged QRS-complexes relative to wildtype littermates (WT). Cx30.2-deficient mice ($Cx30.2^{LacZ/LacZ}$) developed shorter PQ-intervals as compared to WT, $Cx40^{-/-}$ or Cx30.2/Cx40 double deficient mice. Intracardiac evaluation of the atria-His (AH) and His-ventricle (HV) intervals representing supra and infra-Hisian conduction yielded significant acceleration of supra-Hisian conductivity in $Cx30.2^{LacZ/LacZ}$ (AH: 28.2 ± 4.3 ms) and prolongation of infra-Hisian conduction in $Cx40^{-/-}$ mice (HV: 13.7 ± 2.6 ms). These parameters were unchanged in the $Cx30.2^{LacZ/LacZ}Cx40^{-/-}$ mice (AH: 37.3 ± 5.5 ms, HV: 11.7 ± 2.6 ms), which exhibited AV-nodal and ventricular conduction times similar to WT animals (AH: 35.9 ± 4.4 ms, HV: 10.5 ± 1.9 ms). We conclude that the remaining Cx45 gap junctional channels are sufficient to maintain electrical coupling and cardiac impulse propagation in the AV-node and proximal ventricular conduction system in mice. We suggest that Cx30.2 and Cx40 act as counterparts in the AV-node and His-bundle, decreasing or increasing, respectively, electrical coupling and conduction velocity in these areas.

1. Introduction

In the mammalian heart, gap junction channels mediate electrical and metabolic communication between adjacent cardiac myocytes. They form intercellular conduits that permit free diffusion of molecules of <1.8 kDa molecular mass, such as ions, metabolites and small peptides [1,2]. This electrical coupling facilitates directed and organized impulse propagation necessary for the coordinated contraction of the cardiac working myocardium [3]. The protein subunits of gap junctional channels are termed connexins. These proteins are encoded by a multigene family comprising twenty different genes in mice [4]. In the mouse heart, four connexins, i.e. Cx43, Cx40, Cx45 and Cx30.2, are differently expressed and partially co-expressed in specialized areas. Working myocytes of atria and ventricles as well as cardiomyocytes of the Purkinje fiber network express Cx43, the major cardiac connexin [5–7]. Cx40 has been detected in atrial working myocytes and, in addition, in the central AV-node as well as ventricular conduction system [8,9]. Cx45 is predominantly expressed throughout the whole cardiac conduction system, i.e. sinoatrial (SA) node, AV-node including its posterior extension, AV-bundle, bundle branches and proximal Purkinje fibers [10–12]. Controversial reports have been published about the expression of Cx45 in working cardiomyocytes [11]. We recently demonstrated that Cx30.2 is strongly expressed in the SA-node, AV-node with posterior extension and to a lower extent in the His-bundle and bundle branches [13,14]. In these areas extensive co-expression of Cx30.2 and Cx45 within the same junctional plaques was detected, whereas Cx30.2 and Cx40 expression rarely overlapped [15].

During the last years the functional role of different cardiac connexins was investigated with ubiquitous and cardiomyocyte-specific null mutant mice, which demonstrated that Cx43 and Cx45 are indispensable for cardiac development and function [7,12]. In contrast, ablation of Cx40 or Cx30.2 in the mouse heart resulted in

* Corresponding author. Tel.: +49 228 287 5507; fax: +49 228 287 4053.
E-mail address: jan.schrickel@ukb.uni-bonn.de (J.W. Schrickel).
[1] Both first authors contributed equally to this work.
[2] Both senior authors contributed equally to this work.

0022-2828/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2009.02.012

milder phenotypes not impeding survival, thus allowing the detailed investigation of their contribution to cardiac impulse propagation in adult null mutants. Cx40 deficient mice exhibited slower conduction velocity in the atria, decreased AV-conduction as well as impaired conduction in the bundle branches due to the removal of high conductance Cx40 channels in these regions [8,9,16]. In contrast, ablation of Cx30.2 resulted in an acceleration of AV-nodal impulse transition and higher ventricular response rates during induced episodes of atrial fibrillation [13]. Therefore, we suggested that Cx30.2 containing gap junctional channels in the mouse heart contribute to the deceleration of conduction in the AV-node, which is necessary for the coordination of atrial and ventricular contraction and facilitates its protective filter function in pathophysiological states such as atrial tachyarrhythmias.

However, it has not been explored to what extent the slow conducting Cx30.2 and fast conducting Cx40 containing channels might fulfill redundant or opposite roles in electrical coupling, especially in regions of putative co-expression. In the present study we generated Cx30.2/Cx40 double deficient mice ($Cx30.2^{LacZ/LacZ}$ $Cx40^{-/-}$) in order to compare their cardiac electrical activity to mice deficient in only Cx30.2 ($Cx30.2^{LacZ/LacZ}$) or Cx40 ($Cx40^{-/-}$). Intracardiac electrograms revealed that the ablation of both connexins resulted in milder occurrence or loss of the alterations in AV-nodal conduction observed after deletion of single connexin isotypes. The remaining Cx45 appears to provide coupling for the transmission of electrical impulses through the AV-node and proximal ventricular conduction system in these double deficient animals. Furthermore, our results indicate that Cx30.2 and Cx40 mainly act as counterparts, possibly responsible for the fine tuning of AV-nodal conduction velocity in the mouse heart.

2. Materials and methods

2.1. Generation and genotyping of mice

Mice were maintained under 12 h/12 h dark/light cycle, provided with water and food ad libitum. The animals were raised and kept according to federal and local legislation for animal welfare in Germany. The investigations also conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health and the *Position of the American Heart Association on Research Animal Use* (AHA, Nov. 11, 1984).

Cx30.2 deficient mice expressing LacZ reporter coding DNA instead of Cx30.2 coding DNA ($Cx30.2^{LacZ/LacZ}$) and Cx40 deficient mice ($Cx40^{-/-}$) were generated as previously described [8,13]. For the generation of ($Cx30.2^{LacZ/LacZ}Cx40^{-/-}$), $Cx30.2^{LacZ/LacZ}$ mice and $Cx40^{-/-}$ mice were interbred to obtain double heterozygous offspring ($Cx30.2^{+/LacZ}Cx40^{+/-}$). These mice were mated to each other to obtain the following genotypes used for immunocytochemical, morphological and electrophysiological analyses: $Cx30.2^{+/+}Cx40^{+/+}$, $Cx30.2^{LacZ/LacZ}Cx40^{+/+}$, $Cx30.2^{+/+}Cx40^{-/-}$ and $Cx30.2^{LacZ/LacZ}Cx40^{-/-}$. To determine the different Cx30.2 and Cx40 alleles, animals were genotyped by PCR analyses as previously described [13,17] (Fig. S1).

2.2. Histomorphology

Adult mouse hearts were fixed at 4 °C in 4% PFA, dehydrated in a graded series of ethanol and embedded in Paraplast plus (Sherwood Medical Co., St. Louis, MO, USA). Paraffin sections (3 μm) were stained with hematoxylin-eosin.

2.3. Immunocytochemical analyses

Adult hearts from mice of different genotypes were frozen in the gas phase of liquid nitrogen and cryosectioned (12 μm). Subsequently heart cryosections were fixed in 4% paraformaldehyde (PFA) for 10 min, washed three times, digested with a 1:100 dilution of Rnase2000 (Roche) in washing buffer (50 mM Tris, 1.5% NaCl, 0.3% Triton X-100, pH 7.6), washed again and incubated for 1 h at room temperature in blocking solution (washing buffer containing 5% NGS (normal goat serum) and 5% BSA (bovine serum albumine). Slides were incubated with the polyclonal antibodies anti-Cx30.2 (1:250), anti-Cx40 (1:250, Alpha Diagnostics), anti-Cx43 (1:500) [18] or anti-Cx45 (1:500) [19] overnight at 4 °C. Connexins were visualized using fluorochrome-conjugated secondary antibodies, Alexa(488)-goat anti-rabbit (1:1000). Nuclei were stained with propidium iodide. Slides were washed and cover slips were mounted with Permafluor aqueous mounting medium (Beckman). Fluorescent and bright field images were recorded using a Zeiss confocal (LSM 510) microscope.

2.4. Echocardiography

High resolutional mouse echocardiography was performed using a commercially available ultrasound system (HDI-5000, Philips Medical Systems, Bothell, WA, USA) equipped with a linear array transducer (15 MHz, harmonic-mode). Adult mice were investigated during inhalative anaesthesia (1.5–1.8 vol.% sevoflurane in 50% N_2O/50% O_2) at constant body temperature (37 °C) as previously described [20,21]. Briefly, left-ventricular volumes at end-diastole and end-systole, left-ventricular mass and ejection fraction (EF) were calculated using the area-length method. Parasternal short-axis views were visually divided into six segments by the investigator. End-diastolic measurements were performed at the peak of the R-wave, whereas end-systolic measurements were assessed at the time of minimum internal chamber dimensions.

2.5. Electrophysiological investigation (EPI) and surface-ECG

18 adult WT, 10 $Cx30.2^{LacZ/LacZ}$, 12 $Cx40^{-/-}$ and 10 $Cx30.2^{LacZ/LacZ}$ $Cx40^{-/-}$ mice were in vivo electrophysiologically examined using a single catheter technique as described before [13,22,23]. In brief, preparation, catheterization, and electrophysiological investigations were performed under inhalation anaesthesia (induction period 2.5 vol.%, maintenance 1.0 vol.% isoflurane in 70% N_2O/30% O_2). A surface 6-lead-ECG was monitored continuously and standard ECG-parameters were analyzed under stable baseline conditions (Fig. S2A).

Table 1
Surface-ECG and standard electrophysiological parameters.

Surface-ECG	WT ($n=18$)	$Cx40^{-/-}$ ($n=12$)	$Cx30.2^{LacZ/LacZ}$ ($n=10$)	$Cx30.2^{LacZ/LacZ}$ $Cx40^{-/-}$ ($n=10$)
Heart Rate (bpm)	445 ± 59	490 ± 91	451 ± 83	421 ± 40
P (ms)	15.6 ± 1.8	20.9 ± 5.9*	14.4 ± 1.9	19.2 ± 3.9*
PQ (ms)	46.6 ± 4.0	49.5 ± 4.7*	35.7 ± 3.4**	51.6 ± 5.1*
QRS (ms)	12.9 ± 1.9	19.5 ± 3.1*	14.2 ± 2.0	19.9 ± 2.4*
QT (ms)	37.3 ± 4.3	39.5 ± 6.3	37.8 ± 3.0	43.8 ± 3.3*
Intracardiac testing				
AH	35.9 ± 4.4	34.4 ± 2.3	28.2 ± 4.3***	37.3 ± 5.5
HV	10.5 ± 1.9	13.7 ± 2.6*	9.7 ± 1.2	11.7 ± 2.5
SNRT (ms)	184 ± 49	184 ± 46	176 ± 33	178 ± 36
WBP (ms)	86.6 ± 8.3	78.9 ± 5.5	79.7 ± 7.2**	81.5 ± 6.7
ARP (ms)	24.3 ± 12.2	20.6 ± 5.6	23.4 ± 6.3	21.1 ± 4.2
AVNRP (ms)	48.8 ± 7.9	44.4 ± 8.1	49.1 ± 12.8	52.5 ± 10.1
VRP (ms)	30.3 ± 5.3	37.2 ± 5.7	33.2 ± 10.1	32.0 ± 9.2

All data presented are mean ± standard deviation. $P \leq 0.05$ was regarded as statistically significant. bpm, beats per minute. AH: interval from the start of A to maximum of His (H) signal; HV: Max. of H to start of V signal; SNRT: sinus-node recovery-time at S1S1: 120 ms; WBP: Wenckebach-periodicity; ARP: atrial refractory period; AVNRP; AV-nodal refractory period at S1S1: 120 ms; VRP: ventricular refractory period at S1S1: 120 ms.

* $P<0.05$ versus WT and $Cx30.2^{LacZ/LacZ}$.

** $P<0.05$ versus WT.

*** $P=0.05$ versus WT, $Cx40^{-/-}$ and $Cx30.2^{LacZ/LacZ}Cx40^{-/-}$.