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Original article

The Connexin40A96S mutation from a patient with atrial fibrillation causes decreased atrial conduction velocities and sustained episodes of induced atrial fibrillation in mice

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ABSTRACT

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia and a major cause of stroke. In the mammalian heart the gap junction proteins connexin40 (Cx40) and connexin43 (Cx43) are strongly expressed in the atrial myocardium mediating effective propagation of electrical impulses. Different heterozygous mutations in the coding region for Cx40 were identified in patients with AF. We have generated transgenic Cx40A96S mice harboring one of these mutations, the loss-of-function Cx40A96S mutation, as a model for atrial fibrillation. Cx40A96S mice were characterized by immunochemical and electrophysiological analyses. Significantly reduced atrial conduction velocities and strongly prolonged episodes of atrial fibrillation were found after induction in Cx40A96S mice. Analyses of the gating properties of Cx40A96S channels in cultured HeLa cells also revealed significantly lower junctional conductance and enhanced sensitivity voltage gating of Cx40A96S in comparison to Cx40 wild-type gap junctions. This is caused by reduced open probabilities of Cx40A96S gap junction channels, while single channel conductance remained the same. Similar to the corresponding patient, heterozygous Cx40A96S mice revealed normal expression levels and localization of the Cx40 protein. We conclude that heterozygous Cx40A96S mice exhibit prolonged episodes of induced atrial fibrillation and severely reduced atrial conduction velocities similar to the corresponding human patient.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia among humans and the risk to develop atrial fibrillation increases with progressing age of patients. The disease is characterized by spontaneous, irregular and chaotic electrical activation of the atrial myocardium which can, in the case of fast atrio-ventricular conduction, cause tachycardiomoypathy and heart failure. Furthermore, patients suffering from atrial fibrillation exhibit a 6-fold higher risk to develop stroke [1] due to an increased likelihood for clot formation in the left atrial appendage.

In the mammalian heart, the electrical coupling of cardiomyocytes is mediated by gap junctional proteins, which enable effective propagation of electrical activation and contraction of cardiac myocytes. Gap junctions are transmembranal channel proteins, which allow the exchange of molecules up to a molecular mass of 1.8 kDa between neighboring cells [2]. Gap junction conduits consist of two hemichannels, each composed of six connexin (Cx) protein subunits. Connexins are members of a multigene family, including 20 isoforms in the mouse and 21 members in the human genome [3]. In the mammalian atrium mainly connexin43 (Cx43) and connexin40 (Cx40) are expressed [4]. Cx40 deficient mice show delayed atrial and atrioventricular conduction velocities, as well as an increased vulnerability to atrial arrhythmias [5–7].

In the last years, different heterozygous mutations in the coding region for Cx40 were found in patients with atrial fibrillation [8–13]. The

Abbreviations: AF, atrial fibrillation; Cx, connexin; Cx40A96S, mutation in the connexin40 sequence that leads to the exchange of amino acid residue alanine with serine at position 96 of the Cx40 peptide sequence; GJ, gap junction; ED, embryonic day; PD, postnatal day; CV, conduction velocity; ECG, electrocardiogram; SAECG, standard average ECG recording; EAM, epicardial activation mapping; EPI, electrophysiological investigation; WBP, Wenckebach periodicity; ARP, atrial refractory period; AVNRP, AV-nodal refractory period; VRP, ventricular refractory period; g_j, junctional conductance.

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heterozygous Cx40 mutations identified by Gollob et al. [8], included three somatic and one germ-line mutation, the latter of which leads to the exchange of an alanine with a serine residue at position 96 of the Cx40 protein (Cx40A96S) resulting in non-functional Cx40 gap junction (GJ) channels [8]. Recently, the Cx40A96S mutation was reported in another human patient with lone AF [11]. Besides Cx40, also somatic Cx43 mutations have been identified in human patients with lone AF [14]. We generated a transgenic mouse line harboring the Cx40A96S mutation in the mouse Gja5 gene (coding for Cx40) as a model for atrial fibrillation.

We have previously described that mice expressing the loss-of-function Cx40A96S variant exhibit renin dependent hypertension [15]. Interestingly, at the time of assessment, at age 61, the corresponding patient with AF was being treated for hypertension with no sign of diabetes or hyperlipidemia (M. Gollob, personal communication, 2010). Taken together this suggests, that loss-of-function mutations in the GJA5 gene can lead to idiopathic atrial fibrillation and renin dependent hypertension in mice and probably men. Other groups have described a point mutation in the coding region of the pore-forming subunit of a potassium channel as molecular cause in a case of familiar atrial fibrillation [16]. Moreover, specific polymorphisms in the regulatory promoter region of the human GJA5 gene, coding for the Cx40 protein, have been associated with both, atrial fibrillation and hypertension [17–19].

The Cx40A96S expressing mouse model offers the possibility to investigate the etiopathology of certain cases of genetically caused human atrial fibrillation and to learn more about the underlying mechanisms.

2. Material and methods

2.1. Treatment of mice

Generation of nonconditional Cx40A96S mice was described previously [15]. Homozygous Cx40A96S mice were obtained by interbreeding heterozygous Cx40A96S mice. All animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health and were approved by the local veterinarians. Mice were kept under standard housing conditions with a 12-h/12-h dark/light cycle, with food and water ad libitum. All invasive procedures (electrophysiological examination, preparation of hearts for Langendorff-perfusion, implantation of Holter ECGs) were conducted under conditions of inhalative anesthesia with the animal breathing spontaneously (induction period 2.5 vol.%, maintenance 1.0–1.2 vol.% isoflurane in 70% N_2O/30% O_2). A constant body temperature of 37 °C was warranted by use of a heating pad.

2.2. Holter ECG: chip implantation and long-term ECG recording

For evaluation of standard ECG parameter under normal conditions in the absence of anesthetic agents and for detection of spontaneous arrhythmias, telemetric ECG recordings were performed in wake mice. For this, telemetric Holter ECG chips with two subcutaneous electrodes (Data Science International, St. Paul, USA) were implanted, using the anesthesia protocol as described above. Chips were implanted under sterile conditions on the upper back of the mouse after medial incision and subcutaneous preparation. ECG leads were subcutaneously implanted in the left-lateral and anterior part of the thorax, and connected to the telemetric chip device. After that, the skin was sutured. Recordings were performed in all mice that showed normal behavior 7–10 days after implantation of the telemetric chips. Recordings were performed for 8 h during day and night in each mouse, electrograms were recorded continuously during this period and sampled and stored digitally using standard mouse Holter equipment (PowerLab™ System, ADInstruments, Milford, MA, USA). Analyses included standard average ECG recording (SAECG). 100 QRS complexes every hour of recording for each mouse were averaged, resulting in 16 analyzable SAECG recording per investigated mouse. Moreover, all recordings were manually screened for spontaneous tachy- and bradyarrhythmias.

2.3. In vivo electrophysiological investigation (EPI)

A surface 6-lead-ECG was obtained by cutaneous clips on each limb. Access to the jugular vein was achieved by lateral cervical incision under sterile conditions. The jugular vein was dissected from surrounding tissue and proximally ligated. A 2-French octapolar mouse-electrophysiological catheter [eight 0.5 mm circular electrodes; electrode pair spacing 0.5 mm (Ciber Mouse, NuMED Inc., New York, NY, USA)] was positioned transveneously in the right heart chambers under constant monitoring of the intracardiac electrograms. Atrial (proximal electrode pairs) and ventricular (distal electrode pairs) electrograms were documented as previously described [7,20–22]. All data were sampled at 4 kHz (Bard stamp amplifier; Bard LabSystem, C.R. Bard Inc., New Jersey, USA) and digitally stored on optical disk.

Transveneous atrial and ventricular recording and stimulation were conducted for electrophysiological investigation. Bipolar electrograms were obtained from each pair of electrodes. For evaluation of supra- and infra-Hisian conduction, AH- and HV-intervals were analyzed in the intracardiac recording in which the His (H) was visible. AH as surrogate for supra-Hisian conduction time was defined as the period from the starting point of the atrial (A) signal to the maximum deflection of the H signal in the intracardial electrogram. HV was defined as the time-period from the maximum deflection of the H signal to first deflection of the QRS-complex in surface lead II (V = ventricular). Pacing threshold currents at 1 ms stimulus duration were 0.63 ± 0.19 mA at atrial and 0.41 ± 0.11 mA at ventricular level. Twice pacing threshold rectangular stimulus pulses were administered by a modified, multi-programmable stimulator with S1S1 stimulus cycle lengths (CLs) down to a stimulus duration of 10 ms (Model 5328; Medtronic, Minneapolis, MN, USA). Pacing maneuvers and analyses of standard electrophysiological parameters were performed as in previous investigations [20–22]. In short, sinus node recovery time (SNRT), Wenckebach-periodicity (WBP), atrial and AV-nodal refractory period (ARP, AVNRP) were evaluated, using fixed rate and extrastimulus atrial pacing. Ventricular refractory period (VRP) was tested by programmed ventricular stimulation with single extrastimulus. Atrial and ventricular programmed and burst stimulation protocols were applied for testing inducibility of atrial fibrillation and ventricular arrhythmias, as previously described [20–22]. In summary, atrial and ventricular burst stimulations were performed for 5 s (S1S1: 50 ms–10 ms, 10 ms stepwise reduction; stimulus amplitudes 1.0 and 2.0 mA) [22,23]. AF was defined as rapid and fragmented atrial electrograms with irregular AV-nodal conduction for >1 s [22]. Number of atrial fibrillation (AF) episodes, mean ventricular heart-rate during AF and duration of AF were analyzed. For analyses of atrial fibrillation episodes, overall inducibility per group, inducible episodes per animal and episode-durations were analyzed.

2.4. Langendorff-perfused hearts and epicardial mapping

For investigation of myocardial conduction velocities and homogeneity of conduction, hearts were Langendorff-perfused and epicardial activation mapping (EAM) using a 128-electrode array was performed [22]. For this, hearts were excorporated and dissected from surrounding tissue in ice-cold Krebs–Henseleit buffer. Following cannulation of the aorta, the heart was immersed in a water-jacketed chamber and further fixed on a moisturized support. Hearts were then retrogradely perfused in a Langendorff-apparatus (Radnoti Technologies Inc., Monrovia, CA, USA) at constant pressure perfusion (80 mm Hg, resulting in coronary flow between 2 and 2.5 ml/min). The perfusate composition was (in mM): NaCl 110, KCl 4.6, $MgSO_4$ 1.2, CaCl 2, NaH_2PO_4 2, $NaHCO_3$ 25, glucose 8.3, Na-pyruvate 2 and gassed with carbogen (O_2 95%, CO_2 5%), pH, 7.35–7.45 at constantly 37 °C. 128 unipolar electrograms (interelectrode distance: 130 ± 7 μm) were recorded from the epicardial surface of