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Neuromuscular Disorders ■■ (2015) ■■–■■

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Abnormal sodium current properties contribute to cardiac electrical and contractile dysfunction in a mouse model of myotonic dystrophy type 1

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Received 21 October 2014; received in revised form 14 November 2014; accepted 24 November 2014

Abstract

Myotonic dystrophy type 1 (DM1) is the most common neuromuscular disorder and is associated with cardiac conduction defects. However, the mechanisms of cardiac arrhythmias in DM1 are unknown. We tested the hypothesis that abnormalities in the cardiac sodium current (I_{Na}) are involved, and used a transgenic mouse model reproducing the expression of triplet expansion observed in DM1 (DMSXL mouse). The injection of the class-I antiarrhythmic agent flecainide induced prominent conduction abnormalities and significantly lowered the radial tissular velocities and strain rate in DMSXL mice compared to WT. These abnormalities were more pronounced in 8-month-old mice than in 3-month-old mice. Ventricular action potentials recorded by standard glass microelectrode technique exhibited a lower maximum upstroke velocity $[dV/dt]_{max}$ in DMSXL. This decreased $[dV/dt]_{max}$ was associated with a 1.7 fold faster inactivation of I_{Na} in DMSXL myocytes measured by the whole-cell patch-clamp technique. Finally in the DMSXL mouse, no mutation in the *Scn5a* gene was detected and neither cardiac fibrosis nor abnormalities of expression of the sodium channel protein were observed. Therefore, alterations in the sodium current markedly contributed to electrical conduction block in DM1. This result should guide pharmaceutical and clinical research toward better therapy for the cardiac arrhythmias associated with DM1.

Keywords: Steinert disease; Myotonic dystrophy type 1; Conduction disorders; Transgenic mice; Electrophysiology; Arrhythmia basic study; Sodium current channelopathy

1. Introduction

Myotonic dystrophy type 1 (DM1), also known as Steinert's disease, is the most common inherited neuromuscular disease in adults [1]. DM1 is a genetic disorder caused by the expansion of triplet repeats (CTG)n in the 3′ region of the *DMPK* gene [2]. The main mechanism leading to phenotypical abnormalities of DM1 is the nuclear accumulation of mutant mRNAs causing aberrant alternative splicing of multiple pre-mRNAs [3,4]. Cardiac electrical disturbances such as atrioventricular conduction blocks represent the most frequent cardiac manifestation of DM1 with major impacts on clinical outcomes, as up to 1/3 of patients die suddenly [5–7]. Given the impossibility to perform large experimental studies using human myocardium of patients with DM1, animal models are crucial to underpin the pathophysiology of this disease. In mouse models of DM1, conduction disturbances appeared a few days after inducing the expansion of the CTG triplet [8–10]. These studies point to alterations in cardiac conduction and excitability properties as an early event in the constitution of DM1 associated cardiomyopathies. However, mechanisms linking constitutive CTG expansion and cardiac phenotypes in DM1 patients are not known. Recently, our team published that Brugada syndrome could be potentially implicated in

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http://dx.doi.org/10.1016/j.nmd.2014.11.018
0960-8966/

ventricular tachyarrhythmias and sudden death in a large cohort of 914 DM1 patients [11]. Another recent publication by Pambrun et al. reported that DM1 cardiomyopathy mimics and exacerbates Brugada phenotype induced by a loss of function of the Nav1.5 sodium channel mutation [12]. Therefore, exploring the sodium current (I_{Na}) appeared promising to explain the cardiac electrical disturbances in DM1.

The present study was undertaken to examine the mechanisms underlying the cardiac phenotype of DM1 using a mouse model that constitutively expresses the human DM1 locus under the regulation of its own promoter and its *cis* regulatory elements, the DMSXL mouse model. The originality of this model lies in the pattern of expression of the *DMPK* gene in DMSXL which is similar to DM1 patients including a high level of expression in the heart [13]. This model reproduces the main clinical characteristics observed in the human disease including reduced muscle strength, lower motor performances, peripheral neuropathy and respiratory impairments [13–15]. However, nothing is known about the cardiac phenotype of the DMSXL mouse.

Using a combination of physiological and pharmacological approaches, we provide evidence for reduced cellular excitability of the ventricular myocardium in the DMSXL mouse which appear to be due to anomalies related to the sodium current (I_{Na}) and which could account for the electrical and contractile dysfunctions seen in DM1 cardiopathy.

2. Materials and methods

2.1. DMSXL transgenic mouse model of DM1

The 45-kb genomic fragment carrying the *dystrophia myotonica* protein kinase gene from a DM1 locus patient was used to create homozygous DMSXL transgenic mouse model with >1000 cytosine-thymine-guanine repeats expansion [16]. DMSXL mice show the highest level of expression of CTG repeats and nuclear *DMPK* mRNA foci in cardiac and skeletal muscles [16,17]. Housing and handling of mice were performed in accordance with the guidelines established by the *French Council on Animal Care "Guide for the Care and Use of Laboratory Animals": EEC86/609* Council Directive – Decree 2001-131.

2.2. Electrocardiographic measurements

We anesthetized 3- and 8-month-old WT and DMSXL male mice with an intraperitoneal injection (i.p.) of pentobarbital (20 mg/kg ip). The surface ECG was recorded using 23-gauge, subcutaneous Gould RS 3200 needle electrodes (Gould Instrument Systems) affixed to each limb. ECG was continuously monitored for 5 min before (baseline) and 16 min after the injection of the antiarrhythmic drug flecainide acetate, 20 mg/kg body weight, i.p. Indeed, class I antiarrhythmic drugs are used as a pharmacological stress testing of conduction disorders in humans [18] . Moreover, flecainide test has been used in transgenic mice with mutated cardiac sodium channel to reveal concealed conduction disorders [19,20]. Heart rate and PQ, QRS, and QT duration were measured manually. The values obtained from 5 consecutive cardiac cycles were averaged. The QT interval was corrected for heart rate, using the modified Bazett formula, $QTc = QT/(RR/100)^{0.5}$. Since second or third degree atrioventricular blocks are characterized by the dissociation between atria and ventricles depolarization, the PR interval which reflects the atria to ventricles conduction time was not measurable in flecainide condition.

2.3. Conventional and Doppler Tissue Imaging echocardiography

Echocardiographic images were acquired in 3-month-old and 8-month-old WT and DMSXL mice, using a 13-MHz linear-array transducer with a digital ultrasound system (Vivid 7, GE Medical Systems). Mice were lightly sedated with an i.p. of ketamine 80 mg/kg. Acquisitions were carried out before and 16 min after the injection of flecainide acetate, 20 mg/kg body weight, i.p.

Standard echocardiography included the measurements of left ventricular (LV) end diastolic diameter, interventricular septal thickness, LV ejection fraction and mitral E wave. Tissue Doppler images were collected with the use of parasternal short-axis views at the midventricular level, at a frame rate of 483 frames per second and a depth of 1 cm. The Nyquist limit of the velocity was 15 cm/s, with a pulse repetition frequency of 2.5 kHz. TDI analysis was performed offline with the use of a customized version of the EchoPac Software (GE Medical). Myocardial velocities were computed from a region of interest (0.2×0.2 mm) that was manually positioned along the posterior wall on the endocardial and epicardial surfaces to measure the peak systolic endo (V_{endo}) and epicardial (V_{epi}) velocities, respectively, as previously described [21]. Radial strain rate (SR) was then measured over an axial distance of 1 mm (width 0.6 mm). A tissue Doppler frequency of 6.4 MHz was used. The temporal smoothing filters were turned off for all measurements. Time–velocity and time–SR plots were obtained, and peak systolic velocities and SR were measured. The values obtained in 5 consecutive cardiac cycles were averaged. The echocardiographic data were compared only if the heart rates did not differ among the two groups.

2.4. Patch-clamp experiments

Ventricular myocytes were isolated by the Langendorff perfusion technique as previously described [22]. Sodium current (I_{Na}) was recorded by the whole-cell patch-clamp technique in freshly isolated cardiomyocytes from 8-month-old WT and DMSXL mice at a room temperature of 22–25 °C. Sodium current density–membrane potential (V_m) relationships were calculated and peaks of the current were compared at −20 mV. The activation–V_m and availability–V_m relationships were also determined. Ionic currents were recorded by the whole-cell patch-clamp technique with an Axopatch™ 200B patch-clamp amplifier (Axon Instruments, Molecular Devices). The resistance of the Corning® Kovar Sealing code 7052, WPI patch pipettes (Glass Dynamics) ranged between 1 and 2 MΩ. The currents were filtered at 20 kHz (−3 dB, 8-pole low-pass Bessel filter) and digitized at 50 kHz using a Digidata 1200 acquisition system (Axon Instruments). The series resistance

Please cite this article in press as: Vincent Algalarrondo, et al., Abnormal sodium current properties contribute to cardiac electrical and contractile dysfunction in a mouse model of myotonic dystrophy type 1, Neuromuscular Disorders (2015), doi: 10.1016/j.nmd.2014.11.018