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Altered β-adrenergic response in mice lacking myotonic dystrophy protein kinase (DMPK)

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Abstract

The protein kinase product of the gene mutated in myotonic dystrophy 1 (DMPK) is reported to play a role in cardiac pathophysiology. To gain insight into the molecular mechanisms modulated by DMPK, we characterize the impact of DMPK ablation in the context of cardiac β-adrenergic function. Our data demonstrate that DMPK knock-out mice present altered β-agonist-induced responses and suggest that this is due, at least in part, to a reduced density of β1-adrenergic receptors in cardiac plasma membranes.

Keywords

β-adrenergic; DMPK; myotonic dystrophy; isoproterenol

Introduction

Myotonic muscular dystrophy type 1 (DM1) is an autosomal, dominant inherited, neuromuscular disorder [1]. The DM1 mutation is an unstable CTG repeat expansion in the 3′-untranslated region of DMPK gene [2-4]. Cardiovascular disease is one of the most prevalent causes of death in DM1 patients [1]. Nuclear accumulation of $(CUG)_n$-DMPK transcripts plays a key role in the manifestation of many of DM1 cardiac symptoms through a detrimental impact on a set of cellular pathways that regulate mechanisms of alternative splicing [5-7]. Moreover, DM1 subjects present low abundance of DMPK protein in heart and skeletal muscle [8, 9] which suggests that DMPK insufficiency may represent a concomitant mechanism for disease expression. Indeed, DMPK knock-out (KO) mice recapitulate many DM1 cardiac conduction defects [10–11]. Some DM1 cases present a dysfunction in the autonomic nervous system which has been considered a risk factor for cardiac abnormalities [12]. However, no specific aberrant mRNA splicing related to adrenergic signaling has been reported in DM1 models or tissues.

DMPK mediates the translocation of insulin and IGF-1 receptors to the plasma membrane (PM) [13] and it has been proposed that it plays a role in the regulation of intracellular trafficking of membrane proteins [14]. Here we report the characterization of the impact of DMPK ablation in the context of cardiac β-adrenergic function. Our data indicate that

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DMPK is required for β-agonist induced heart rate (HR) adjustment and Ser_{16}-PLN phosphorylation and suggest that these effects are due at least in part to a role of DMPK in the correct targeting of β_1-adrenergic receptors (β_1-AR) to the PM.

Materials and Methods

Mouse experiments

All animal studies were performed in accordance with the guidelines and with the approval of the Institutional Review Committee for Animal Care (University of Barcelona and Sanford-Burnham Institute). *Dmpk*$^{+/-}$ mice on 129SV background were generated by Reddy *et al.* and backcrossed as previously reported [13, 15].

Transthoracic echocardiography

Studies were performed in isoflurane-anesthetized closed-chest mice using a Visual Sonic Vevo 770 fitted with an 8-15 MHz linear array transducer [16]. DMPK$^{-/-}$ (KO) (n=17), DMPK$^{+/-}$ (HET) (n=3) and WT (n=10) mice were administered intravenously with increasing doses of isoproterenol (3-12-30 μg/kg) every 5 minutes and subsequently, HR was measured. Continuous recording was performed at baseline and 45–60 s after each dose of agonist.

Biochemical analyses

Mice were injected intraperitoneally with isoproterenol (2 mg/kg body weight) or saline solution. After 10 minutes, hearts were extracted and freeze-clamped in liquid nitrogen. Tissue was homogenized and immunoblotting analyses were performed as described (n=3-5) [13]. Antibodies used were anti-mouse-DMPK (Zymed) and anti-PLN and anti-phosphoSer_{16}-PLN (Millipore). Specific protein expression levels were quantified by scanning densitometry.

Cardiac PM preparation

Cardiac PMs were prepared by differential centrifugation as described (n=3) [13, 17]. Antibodies were: anti-Na^+/K^+-ATPase (Abcam), anti-β1-AR (Sigma) and anti-EEA1 (BD Transduction Laboratories). Specific protein expression levels were quantified by scanning densitometry and expressed as fold over WT.

Results

In order to evaluate the pathophysiological consequences of DMPK ablation in cardiac adrenergic response, we challenged mice by intravenously administering increasing doses of isoproterenol (3-12-30 μg/kg) and subsequently measuring their HR. WT and HET mice responded to isoproterenol in a dose-dependent manner, by increasing their HR as monitored by echocardiography analysis (Fig. 1 A). In contrast, KO mice did not show a significant response to β-adrenergic agonist.

We further analyzed the role of DMPK in β-agonist responsiveness by examining PLN phosphorylation at Ser_{16} —a well characterized β_1-AR-dependent response— after *in vivo* treatment with isoproterenol. WT mice presented a 4-fold increase in P-Ser_{16}-PLN in response to isoproterenol whereas KO mice did not exhibit a significant response to the β-agonist (Fig. 1 B).

Regarding the molecular mechanism whereby DMPK could influence β-agonist signaling, we analyzed its role in the intracellular traffic of β_1-AR. To this end, we determined the density of β_1-AR in cardiac sarcolemma of WT and KO mice. PM fractions prepared from

whole hearts were verified to be enriched by 2.5-fold in the PM marker Na^+/K^+-ATPase compared to total extracts and were depleted in the intracellular marker early endosome antigen 1 EEA1 (Fig 1, C). In total extracts, Na^+/K^+-ATPase and β_1-AR protein content was similar for WT and KO mice (Fig 1, D and E). In contrast, the protein content of the β_1-AR in the PM fraction was significantly lower in the KO mice (Fig 1, D and E). This effect was specific, as the PM marker Na^+/K^+-ATPase showed similar levels in WT and KO mice both in PM and in total extract fractions (Fig 1, D and E).

Discussion

The role of DMPK in regulating receptor intracellular trafficking that we have previously reported [13, 14] is further reinforced by the results presented here showing that DMPK-KO mice present significantly reduced β_1-AR localization at cardiac sarcolemma without changes in total receptor expression. HeLa cell transfection experiments using DMPK mutants and pEGFP-β_2AR support these data and point to a role for DMPK in the intracellular trafficking of β-ARs (Supplementary Figure 1). The decrease in β_1-AR PM density in the KO mice correlates with the alteration of two well characterized cardiac responses to isoproterenol, HR adjustment and Ser_{16}PLN-phosphorylation. In contrast, DMPK heterozygous mice showed an unaltered HR response to isoproterenol compared with WT mice (Fig. 1 A) and a correct localization of β_1-AR at the PM (data not shown). As DM1 is inherited in an autosomal dominant manner, our data do not conclusively show whether DMPK-dependent β-adrenergic alterations could be involved in the DM1 cardiac phenotype. However, although rare, some cases of homozygous DM1 have been reported [18] and our results may offer important clues for understanding the molecular mechanisms contributing to their phenotype.

Our results are consistent with the reported essential role of β_1-AR in the stress-induced enhancement of cardiac function. Indeed, atrial and ventricular preparations from β_1-AR KO mice failed to show any responsiveness to isoproterenol, while WT preparations showed significant chronotropic and inotropic responses to the β-agonist [19]. Our data reveal a new modulatory role for DMPK in acute catecholaminergic stimulation and may have clinical relevance as alterations in β-adrenergic response have been reported in failing hearts of animal models and humans [20].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DMPK	myotonic dystrophy protein kinase
DM1	myotonic dystrophy type 1
AR	adrenergic receptor
HR	heart rate
PLN	phospholamban
PM	plasma membrane, β_1-AR, β_1-adrenergic receptors

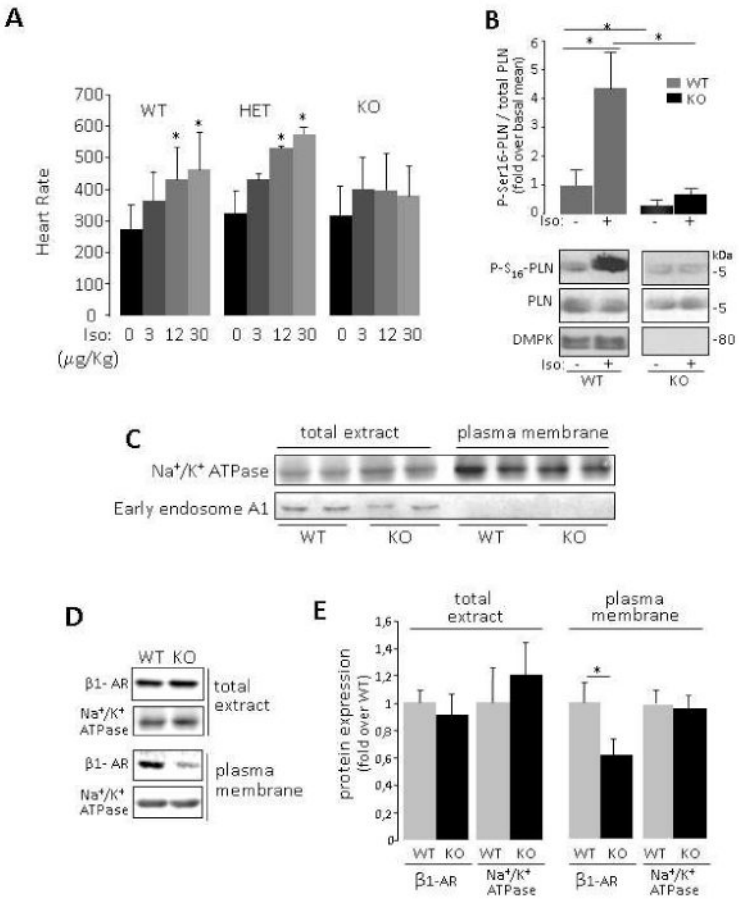

Figure 1. Altered β-agonist responsiveness and decreased plasma membrane density of β1-adrenergic receptors (β1-AR) in hearts from DMPK KO mice

(**A**) DMPK$^{-/-}$ (KO) but not DMPK$^{-/+}$ (HET) mice show an altered heart rate response to isoproterenol. (**B**) DMPK KO mice present altered phospholamban Ser_{16} phosphorylation ($P\text{-}S_{16}$-PLN) after *in vivo* treatment with isoproterenol. (**C**) Cardiac PM fractions were confirmed to be enriched in PM marker Na^+/K^+-ATPase and depleted in the intracellular marker EEA1. (**D, E**) KO and WT mice present similar total expression of Na^+/K^+-ATPase and of β_1-AR. The content of β_1-AR in PM is lower in KO than in WT mice, whereas Na^+/K^+-ATPase is expressed similarly in both groups. (*$p < 0.05$ vs control values).