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Axial stretch-dependent cation entry in dystrophic cardiomyopathy: Involvement of several TRPs channels

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ABSTRACT

In Duchenne muscular dystrophy (DMD), deficiency of the cytoskeletal protein dystrophin leads to well-described defects in skeletal muscle but also to dilated cardiomyopathy (DCM). In cardiac cells, the subsarcolemmal localization of dystrophin is thought to protect the membrane from mechanical stress. The dystrophin deficiency leads to membrane instability and a high stress-induced Ca^{2+} influx due to dysregulation of sarcolemmal channels such as stretch-activated channels (SACs). In this work divalent cation entry has been explored in isolated ventricular Wild Type (WT) and *mdx* cardiomyocytes in two different conditions: at rest and during the application of an axial stretch. At rest, our results suggest that activation of TRPV2 channels participates to a constitutive basal cation entry in *mdx* cardiomyocytes.Using microcarbon fibres technique, an axial stretchwas applied to mimic effects of physiological conditions of ventricular filling and study on cation influx bythe Mn^{2+}-quenching techniquedemonstrated a high stretch-dependentcationic influx in dystrophic cells, partially due to SACs. Involvement of TRPs channels in this excessive Ca^{2+} influx has been investigated using specific modulators and demonstratedboth sarcolemmal localization and an abnormal activity of TRPV2 channels.

In conclusion, TRPV2 channels are demonstrated here to play a key role in cation influx and dysregulation in dystrophin deficient cardiomyocytes, enhanced in stretching conditions.

1. Introduction

The Duchenne muscular dystrophy (DMD) is a severe X-linked disease affecting 1 in 3500 male births. Initially, DMD patients develop progressive weakness and loss the ability to walk around age 10. DMD is also associated by 20 years of age with cardiac complications including dilated cardiomyopathy (DCM) and arrhythmias, causing the death of about 20% of patients [1,2]. Mutations in the dystrophin gene on chromosome Xp21 result in the absence of the 427 kDa cytoskeletal protein dystrophin [3]. In cardiomyocytes, its subsarcolemmal and sarcomeric localization along the T-tubule network may be essential to maintain membrane integrity [4]. This protein is also a key component of the transmembrane dystrophin-associated glycoproteins (DAG) that connects cytoskeleton to the extracellular matrix [5]. It is thought that lack of dystrophin leads to mechanical instability of cell membrane [6] and renders it more susceptible to rupture [7], causing elevated Ca^{2+} influx and increased susceptibility to oxidative stress [8], which activate each other in a vicious cycle that contributes to DMD pathogenesis. Several studies have suggested that the rise in intracellular Ca^{2+} is an important initiating event in the pathogenesis of dystrophic muscle [9–11]. The increased Ca^{2+} entry occurring during activity, particularly during eccentric exercise, may lead to local proteolytic activation of cationic channels and results in a further increase of Ca^{2+} entry [12].

Stretch-activated channels (SACs) are thought to support excessive Ca^{2+} influx observed in dystrophic muscles [13]. SACs are a subcategory of mechanosensitive channels, which can switch from "closed" to "open" state in response to stretch alone or to a direct

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mechanical membrane deformation (see for review: [14]). They are permeable to Na^+, Ca^{2+} and K^+, and have been suggested to be primarily involved in the pathogenesis of DMD [15,16,17]. Cardiac SACs can be either cation non-selective (SAC_{ns}) or potassium-selective (SAC_k). SAC_{ns} are thought to be localized in membrane regions that are difficult to access for patch-clamp studies, such as T-tubules [18], caveolae [19], or intercalated discs [20]. Despite the lack of molecular identification, there are several prominent candidates for mammalian cardiac SACs. Genetic screenings in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophilia* and *Danio rerio* indicated that transient receptor potential (TRP) channels subunits are involved in mechanical sensing [21–23]. TRP channels form a large family of cationic channels that likely function as tetramers in various processes and have been recently recognized as key molecules in pathological cardiac hypertrophy and heart failure [24,25].

Canonical TRP channels (TRPCs) are a group of mammalian Ca^{2+}-permeable channels which mediate store-operated Ca^{2+} entry as well as store-independent Ca^{2+} influx [26–28]. TRPC1 channels are widely expressed in cardiac myocytes and may be located in T-tubules [18] which are consistent with the hypothesized spatial distribution of endogenous SAC_{ns} [18]. In dystrophic fibres, TRPC1 have been involved in abnormal Ca^{2+} influx [29] and Ward et al. [30] have shown that expression level of TRPC1 was increased in aged *mdx*-DCM heart. TRPC1 was also shown to support elevated store-operated calcium entry in dystrophin deficient myotubes and fibres [29,31] as well as Orai1 in FDB fibres [32]. Studies in heterologous system demonstrated that TRPC1 expression induced a 10-fold increase of SAC_{ns} currents and TRPC1 inhibition through RNA antisens reduced these currents [33]. Subsequently, it has been shown that the specific SAC_{ns} blocker, GsMTx-4, blocked the expressed channel [34]. However, it has also been shown that GsMTx-4 could inhibit SACs formed probably with TRPC6 in smooth muscle [35]. TRPC6 is highly expressed in human heart homogenates [36]. This channel seems also to be located in T-tubules and can be translocated to the plasma membrane after stimulation with α_1-agonists [37]. Furthermore TRPC6 channels have been thought to be potential SAC_{ns} in ventricular cells as the related current could be blocked by a TRPC6 antibody [38]. Thus both TRPC1 and TRPC6 are good candidates for mammalian SAC_{ns}.

TPRV2 channels have also been involved in the stretch-dependent responses in different cell types as murine aortic myocytes or cardiomyocytes [39–41]. In a physiological context, these channels have been shown to be involved in the formation and integrity maintenance of intercalated discs and in the mechanotransduction in these particular areas [20]. Moreover, Rubinstein et al. [42] have demonstrated that TRPV2 channels could be involved in the molecular mechanisms of increased cardiomyocyte contractility. Indeed, authors have suggested that these channels may be involved in the sarcoplasmic reticulum (SR) Ca^{2+} load by a small local influx of Ca^{2+} in the vicinity of RyRs. Taking these results into account, TRPV2 seems to be also a good candidate for SAC_{ns}. In a pathological context, they have been demonstrated to be involved in the pathogenesis of myocyte degeneration in dystrophic muscle [43] and to support elevated divalent cation entry in human DMD myotubes [44]. Their sarcolemmal accumulation has been shown in dystrophic ventricular cardiomyocytes in several studies [40,41] suggesting a role in Ca^{2+} dysregulation that can lead to cardiomyocytes death.

Studying SACs is not trivial because of the need of technical skills and experience. Several methods have been developed to perform a mechanical stimulation including the use of glass microneedles [45], suction micropipettes [46] or adhesives [47]. Furthermore, others studies used cell swelling through hypoosmotic shock to investigate SACs activities in dystrophin deficient cardiomyocytes [41,48]. This latter technique leads to cell swelling through water entry, which is necessary for the balancing of ionic gradients between the extracellular medium and cytosolic compartment. The consequent increase of cell volume can provide a three-dimensional stretch of the cell, but changes in volume are not easily related to changes in tension. In the literature, authors agree that it is difficult to distinguish between the effects of the stretch component and other possible effects of swelling, i.e. changes in ions permeability can't be compared with those due to homogenous or local stretch [49].

In this work, divalent cation entry has been explored in isolated ventricular Wild Type (WT) and *mdx* cardiomyocytes in two different conditions: at rest and during the application of an axial stretch. Ventricular cardiomyocytes have been stretched using a carbon microfibers technique [50,51], which has the advantage to achieve an almost homogenous lengthening of the sarcomeres and to best mimic the effect of diastolic filling in physiological conditions [52]. Moreover, the involvement of other channels, as store-operated channels, in the observed Ca^{2+} influx has been investigated with the use of channels blockers.

2. Materials and methods

2.1. Cells isolation

The investigation was conducted in agreement with European Community Council directives as well as with 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).

Ventricular cardiac myocytes were isolated from 10 to 12 months male C57BL10Scsn (WT) mice and C57/BL10ScScn-*mdx* mice (Jackson Laboratory). Mice were euthanized by cervical dislocation, followed by rapid removal of the heart and subsequent enzymatic dissociation of the left ventricular tissue as previously described [53].

2.2. Solutions and chemicals

Before experiments, cells were stored in culture medium containing Dulbecco's modified Eagle's medium (DMEM—Lonza), complemented with 10 μg/mL insulin, 10 μg/mL gentamycin, 4 mM $NaHCO_3$, 10 mM Hepes, 0.2% BSA and 12.5 μM blebbistatin (all from Sigma). During experiments, cardiomyocytes were superfused with Tyrode solution containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.8 mM $MgCl_2$, 10 mM Hepes and 11 mM glucose or with calcium-free Tyrode solution containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $MgCl_2$, 10 mM Hepes, 11 mM glucose, 1 g/L BSA and 20 mM Taurine.

Tranilast (Trn) and TPEN (*N*,*N*,*N*′,*N*′-tetrakis-(2-pyridylmethyl)-ethylenediamine) was purchased from Calbiochem, THC (Δ9-tetrahydrocannabinol) from RTI International, the tarentula toxin GsMTx-4 from Abcam, Streptomycin sulfate (Strp) and 4-methyl-4′-[3.5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-1.2.3-thiadiazole-5-carboxanilide (YM-58483) from Sigma, ryanodine from Merck, fura-2-AM and fluo-8-AM from Santa-Cruz.

2.3. Mechanical stimulation

Axial stretch of single myocyte was performed with the micro-carbon fibres technique previously described [51,52]. Briefly, a pair of carbon fibres (40 μm diameter, GRC—Graphite-reinforced carbon–TMIL—Tsukuba Materials Information Laboratory, Ltd, Tsukuba, Japan) was mounted in glass capillaries, whose thin ends were bent by 30° to ensure near-planar approach to the cell surface. The end of each carbon fibre was attached to the ends of a cardiomyocyte (CF1 and CF2 in Fig. 2A), using two separate three-axis miniature hydraulic manipulators. Axial stretch was performed