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Autonomic, locomotor and cardiac abnormalities in a mouse model of muscular dystrophy: targeting the renin–angiotensin system

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Abstract

Muscular dystrophies are a heterogeneous group of genetic muscle diseases characterized by muscle weakness and atrophy. Mutations in sarcoglycans and other subunits of the dystrophin–glycoprotein complex cause muscular dystrophy and dilated cardiomyopathy in animals and humans. Aberrant autonomic signalling is recognized in a variety of neuromuscular disorders. We hypothesized that activation of the renin–angiotensin system contributes to skeletal muscle and autonomic dysfunction in mice deficient in the sarcoglycan- δ (*Sgcd*) gene at a young age and that this early autonomic dysfunction contributes to the later development of left ventricular (LV) dysfunction and increased mortality. We demonstrated that young *Sgcd*^{−/−} mice exhibit histopathological features of skeletal muscle dystrophy, decreased locomotor activity and severe autonomic dysregulation, but normal LV function. Autonomic regulation continued to deteriorate in *Sgcd*^{−/−} mice with age and was accompanied by LV dysfunction and dilated cardiomyopathy at older ages. Autonomic dysregulation at a young age predicted later development of LV dysfunction and higher mortality in *Sgcd*^{−/−} mice. Treatment of *Sgcd*^{−/−} mice with the angiotensin type 1 receptor blocker losartan for 8–9 weeks, beginning at 3 weeks of age, decreased fibrosis and oxidative stress in skeletal muscle, increased locomotor activity and prevented autonomic dysfunction. Chronic infusion of the counter-regulatory peptide angiotensin-(1–7) resulted in similar protection. We conclude that activation of the renin–angiotensin system, at a young age, contributes to skeletal muscle and autonomic dysfunction in muscular dystrophy. We speculate that the latter is mediated via abnormal sensory nerve and/or cytokine signalling from dystrophic skeletal muscle to the brain and contributes to age-related LV dysfunction, dilated cardiomyopathy, arrhythmias and premature death. Therefore, correcting the early autonomic dysregulation and renin–angiotensin system activation may provide a novel therapeutic approach in muscular dystrophy.

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Additional Information

Competing interests

Drs Sabharwal and Chapleau are collaborating with Tarix Pharmaceuticals in developing novel therapies for muscular dystrophy.

Introduction

The dystrophin–glycoprotein complex forms a physical link between extracellular matrix and intracellular F-actin and stabilizes the membrane during muscle contraction (Ervasti & Campbell, 1993). Sarcoglycans are important subunits of the dystrophin–glycoprotein complex (Barton, 2006). Perturbations in the synthesis and/or positioning of dystrophin–glycoprotein complex proteins cause muscular dystrophies, which are inherited catastrophic neuromuscular disorders characterized by progressive muscle wasting and weakness (Allikian & McNally, 2007). Patients with muscular dystrophy often develop dilated cardiomyopathy and heart failure and, consequently, die prematurely from left ventricular (LV) pump dysfunction or cardiac arrhythmias (Corrado *et al.* 2002).

There is no cure for muscular dystrophy. Current therapeutic strategies include cell-based therapies, gene replacement, mutation-specific treatments and attempts to manage symptoms and complications of the disease (Bushby *et al.* 2009). Aberrant autonomic signalling, e.g. reduced heart rate variability, increased sympathetic-mediated vasoconstriction in exercising muscle and activation of the renin–angiotensin system (RAS), are recognized in human patients and in animal models of muscular dystrophy (Sander *et al.* 2000; Cohn *et al.* 2007; Politano *et al.* 2008). Furthermore, treatment of patients with muscular dystrophy with angiotensin-converting enzyme (ACE) inhibitors and β -blockers has been shown to delay the onset of dilated cardiomyopathy (Duboc *et al.* 2005; Blain *et al.* 2013). These results support our hypothesis that, along with skeletal muscle and cardiac muscle myopathy, neurohumoral dysregulation plays an important role in the pathophysiology of muscular dystrophy (Fig. 1); therefore, correction of the autonomic dysregulation may be a beneficial therapeutic approach in muscular dystrophy.

Autonomic and skeletal muscle dysfunction precedes LV dysfunction in muscular dystrophy

Mutations in the δ -sarcoglycan (*Sgcd*) gene cause limb girdle muscular dystrophy 2F, an autosomal recessive disease. Although sarcoglycanopathy has a relatively low prevalence, understanding its pathogenesis has implications for patients with other types of muscular dystrophy and dilated cardiomyopathy (Fanin *et al.* 1997). Thus, we studied autonomic, cardiovascular, cardiac and skeletal muscle phenotypes in *Sgcd*-deficient (*Sgcd*^{−/−}) mice and compared the results with those of control C57BL/6 mice.

At a young age, *Sgcd*^{−/−} mice exhibited impaired baroreflex control of heart rate (assessed by the sequence technique), reduced vagal tone (change in heart rate following cholinergic blockade with methylatropine), increased cardiac sympathetic tone (change in heart rate following β -adrenergic blockade with propranolol) and increased vasomotor tone (depressor response to the ganglionic blocker chlorisondamine; Sabharwal *et al.* 2009). Autonomic dysfunction was accompanied by fibrosis and centralization of nuclei in skeletal muscle, as well as reduced locomotor activity. These indices progressively worsened with age in *Sgcd*^{−/−} mice, with LV dysfunction (measured by echocardiography) occurring only at older ages in *Sgcd*^{−/−} mice (Sabharwal *et al.* 2009). Importantly, we found that autonomic dysregulation at a young age predicted later development of LV dysfunction and higher

mortality in *Sgcd*^{-/-} mice. Similar observations of early-age skeletal muscle dysfunction, i.e. reduced force, increased fibrosis, centralized nuclei, increased serum creatine kinase, muscle fibre splitting, fatty acid infiltration and reduced locomotor activity, have been reported by other laboratories using different animal models of muscular dystrophy (Duclos *et al.* 1998; Ramaswamy *et al.* 2011).

Angiotensin II-induced skeletal muscle and autonomic dysfunction in young *Sgcd*^{-/-} mice

The RAS is of paramount importance in maintaining cardiovascular homeostasis. A simplified version of the RAS is illustrated in Figure 2. Angiotensin-converting enzyme converts angiotensin I (Ang I) to angiotensin II (Ang II) that acts via angiotensin type 1 (AT₁R) and type 2 receptors (AT₂R). The potentially deleterious actions of Ang II, including increases in vascular tone, fibrosis, oxidative stress and sympathetic nerve activity, are mediated by AT₁R (Matsusaka & Ichikawa, 1997). Angiotensin II is cleaved by angiotensin-converting enzyme 2 (ACE2) to form the heptapeptide angiotensin-(1–7) [Ang (1–7)] that acts via binding to the Mas receptor. Angiotensin-(1–7) has been demonstrated to possess antifibrotic, antiremodelling, antioxidant, sympathoinhibitory and vasodilator properties (Santos *et al.* 2008).

Recent studies have demonstrated that the binding of Ang II to AT₁R contributes to skeletal muscle fibrosis and inflammation in muscular dystrophy, with positive therapeutic responses to treatment with the ACE inhibitor enalapril and AT₁R blocker losartan (Cohn *et al.* 2007; Cozzoli *et al.* 2011). These treatments have focused almost exclusively on muscle as the therapeutic target. The involvement of the Ang II–AT₁R axis in mediating autonomic dysfunction in heart failure and hypertension is well established (Zucker *et al.* 2004; Kudo *et al.* 2009). Administration of AT₁R blockers and antioxidants inhibits sympathetic nerve activity and increases parasympathetic nerve activity, heart rate variability and baroreflex sensitivity (Felder *et al.* 2003). In addition, increased parasympathetic nerve activity exerts powerful anti-inflammatory actions (Rosas-Ballina & Tracey, 2009). In preliminary experiments, we found that circulating levels of Ang I and Ang II were increased in young *Sgcd*^{-/-} mice (R. Sabharwal, O. Domenig & M.W. Chapleau, unpublished observations). Furthermore, Ang I, Ang II and AT₁R expression were increased in skeletal muscle of young *Sgcd*^{-/-} mice, whereas Ang-(1–7) and Mas expression were reduced (R. Sabharwal, O. Domenig & M.W. Chapleau, unpublished observations). These findings led us to investigate the role of increased Ang II in mediating autonomic and skeletal muscle dysfunction in muscular dystrophy.

Subgroups of mice were chronically treated with the AT₁R antagonist losartan in drinking water, beginning at 3 weeks of age (Sabharwal *et al.* 2010). Radiotelemeters were subsequently implanted at 9 weeks of age for measuring blood pressure, heart rate and locomotor activity. Treatment of *Sgcd*^{-/-} mice with losartan decreased mean heart rate and increased locomotor activity without affecting mean blood pressure (24 h averages). Losartan improved autonomic function and reduced oxidative stress, AT₁R expression and fibrosis in skeletal muscle of *Sgcd*^{-/-} mice (Sabharwal *et al.* 2010), consistent with previous reports of activation of the RAS in dystrophic muscle of human patients and animals (Cohn

et al. 2007; Sun *et al.* 2009; Cabello-Verrugio *et al.* 2012). Losartan treatment did not significantly affect any of the measured variables in control mice (Sabharwal *et al.* 2010).

Chronic Ang-(1–7) infusion prevents autonomic and skeletal muscle dysfunction in young *Sgcd*^{–/–} mice

Angiotensin-(1–7), acting via Mas receptors, is a powerful counter-regulatory RAS axis that has been described recently (Santos *et al.* 2008; Ferreira *et al.* 2010). Angiotensin-(1–7) counterbalances the deleterious actions of Ang II (Fig. 2) in a variety of pathological states (Diz *et al.* 2007; Ferreira *et al.* 2010; Shenoy *et al.* 2010). We infused Ang-(1–7) via osmotic minipumps to control and *Sgcd*^{–/–} mice, beginning at 3 weeks of age, for 8–9 weeks. Angiotensin-(1–7) treatment prevented autonomic dysfunction, normalized locomotor activity and reduced skeletal muscle fibrosis and oxidative stress in *Sgcd*^{–/–} mice, without affecting any of the measured variables in control mice (Sabharwal & Chapleau, 2012).

Conclusions and perspectives

Based on our findings and reports by other investigators, we conclude that Ang II-induced autonomic and skeletal muscle dysfunction contributes to the pathogenesis of muscular dystrophy. Furthermore, results from our laboratory indicate that early autonomic dysfunction precedes and predicts the severity of LV dysfunction and mortality in *Sgcd*^{–/–} mice; therefore, treatment of muscular dystrophy with the AT₁R antagonist losartan or with Ang-(1–7) at a young age may improve both autonomic regulation and skeletal muscle function.

We speculate that abnormal sensory nerve and/or cytokine signalling from dystrophic muscle to brain may mediate the autonomic dysregulation in muscular dystrophy (Fig. 3). Activation of both the peripheral (Cohn *et al.* 2007; Cozzoli *et al.* 2011; Cabello-Verrugio *et al.* 2012) and central RAS may contribute. Sensory nerve activation and increased circulating levels of Ang II and cytokines have been linked to central RAS activation, oxidative stress and autonomic dysregulation in heart failure and hypertension (Guggilam *et al.* 2008; Wang *et al.* 2008; O’Callaghan *et al.* 2013; Piepoli & Crisafulli, 2014). Delineating the roles of these mechanisms in muscular dystrophy is a promising area for future investigation.

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New Findings

What is the topic of this review?

This symposium report summarizes autonomic, cardiac and skeletal muscle abnormalities in sarcoglycan- δ -deficient mice (*Sgcd*^{-/-}), a mouse model of limb girdle muscular dystrophy, with emphasis on the roles of autonomic dysregulation and activation of the renin–angiotensin system at a young age.

What advances does it highlight?

The contributions of the autonomic nervous system and the renin–angiotensin system to the pathogenesis of muscular dystrophy are highlighted. Results demonstrate that autonomic dysregulation precedes and predicts later development of cardiac dysfunction in *Sgcd*^{-/-} mice and that treatment of young *Sgcd*^{-/-} mice with the angiotensin type 1 receptor antagonist losartan or with angiotensin-(1–7) abrogates the autonomic dysregulation, attenuates skeletal muscle pathology and increases spontaneous locomotor activity.

Figure 1. Pathophysiology of muscular dystrophy

This schematic diagram demonstrates the occurrence of a vicious cycle between pathological changes occurring in the skeletal muscle and heart muscle and neurohumoral dysregulation that is involved in the pathogenesis of muscular dystrophy.

Figure 2. Simplified version of the renin-angiotensin system

Angiotensinogen is cleaved by renin to form angiotensin I (Ang I), which is cleaved further by angiotensin-converting enzyme (ACE) or chymase to form angiotensin II (Ang II). Angiotensin II exerts its actions via binding to either type 1 (AT₁R) or type 2 receptors (AT₂R). Angiotensin-(1-7) is formed by ACE2 and exerts its protective actions via Mas receptors.

Figure 3. Proposed mechanism of autonomic dysfunction in muscular dystrophy

An imbalance between the Ang II–AT₁R and Ang-(1–7)–Mas axes and increased oxidative stress within skeletal muscle is likely to alter cytokine release and sensory afferent signalling to the brain. As demonstrated in heart failure and hypertension, sustained increases in these inputs may activate the central renin–angiotensin system, leading to neuronal oxidative stress, increased sympathetic nerve activity and decreased parasympathetic nerve activity (Guggilam *et al.* 2008; Wang *et al.* 2008; O’Callaghan & Allen, 2013; Piepoli & Crisafulli, 2014). Abbreviations: ParaSNA, parasympathetic nerve activity; and SNA, sympathetic nerve activity.