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Munc18c provides stimulus-selective regulation of GLUT4 but not fatty acid transporter trafficking in skeletal muscle

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ABSTRACT

Insulin-, and contraction-induced GLUT4 and fatty acid (FA) transporter translocation may share common trafficking mechanisms. Our objective was to examine the effects of partial Munc18c ablation on muscle glucose and FA transport, FA oxidation, GLUT4 and FA transporter (FAT/CD36, FABPpm, FATP1, FATP4) trafficking to the sarcolemma, and FAT/CD36 to mitochondria. In Munc18c$^{-/+}$ mice, insulin-stimulated glucose transport and GLUT4 sarcolemmal appearance were impaired, but were unaffected by contraction. Insulin- and contraction-stimulated FA transport, sarcolemmal FA transporter appearance, and contraction-mediated mitochondrial FAT/CD36 were increased normally in Munc18c$^{-/+}$ mice. Hence, Munc18c provides stimulus-specific regulation of GLUT4 trafficking, but not FA transporter trafficking.

1. Introduction

The clearance of glucose and long chain fatty acids (FA) from the circulation are important regulatory processes for maintaining metabolic homeostasis. Transport of glucose and FA into the cell occur via highly regulated protein-mediated mechanisms involving glucose transporter GLUT4 (cf. [1,2]) and selected FA transporters, including fatty acid translocase (FAT/CD36), plasma membrane associated fatty acid binding protein (FABPpm), and the family of fatty acid transporter proteins (FATP 1–6) (cf. [3]). During muscle contraction FAT/CD36 is also translocated to the mitochondria, contributing to upregulating mitochondrial FA oxidation (cf. [3]).

The dysregulation of both glucose and FA transport, and the trafficking of their transport proteins, are implicated in skeletal muscle insulin resistance (cf. [3]). However, the signaling pathways involved in the translocation of FA transporters to the plasma membrane (PM) are largely unknown, although in muscle FAT/CD36 may share some similarities to the insulin- and contraction-mediated signaling cascades of GLUT4 (cf. [3,4]). However, in cardiac cells, independent signaling mechanisms do exist for GLUT4 and FAT/CD36 [5,6]. Whether GLUT4 and FA transporter trafficking to the PM share similar mechanisms remains to be determined.

The subcellular trafficking of GLUT4 to the PM is far better characterized than for FA transporters. GLUT4 trafficking is a vesicle-mediated process following the soluble N-ethylmaleimide-sensitive factor-attachment protein receptor (SNARE) hypothesis where vesicle associated SNARE (vSNARE) proteins associate with complimentary target SNAREs (tSNARE) at the PM (cf. [7]). Formation of the vSNARE–tSNARE complex enables docking, fusion, and integration of GLUT4 vesicles into the PM. A number of accessory proteins are also involved in the formation of the SNARE complex, including vesicle associated membrane proteins (VAMPs), SNARE-related protein (SNAPs), and syntaxins. These accessory proteins facilitate the complementary binding of vSNARE and tSNARE vesicles, ensuring proper PM insertion of GLUT4. In addition, three homologs (a, b, and c) of Munc18, from the Sec1 protein family are known to be present at the mammalian PM [8,9]. Of these, Munc18c is ubiquitously expressed and has binding specificity for the tSNARE protein

Abbreviations: FA, fatty acid; FAT/CD36, fatty acid translocase; GLUT4, glucose transporter; FABPpm, plasma membrane associated fatty acid binding protein; FATP, fatty acid transport protein; PM, plasma membrane; AMPK, 5′AMP-activated protein kinase; SNARE, soluble N-ethylmaleimide-sensitive factor-attachment protein receptor; SS, subsarcolemmal mitochondria; IMF, intermyofibrillar mitochondria

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syntaxin4 [10]. In skeletal muscle, partial ablation of Munc18c in heterozygous knockout mice (Munc18c$^{-/+}$) induces insulin resistance due to impaired GLUT4 translocation [11].

Munc18c may however only be involved in insulin-stimulated GLUT4 trafficking, since GLUT4 trafficking in insulin resistant muscle is impaired while in the same muscles contraction-stimulated GLUT4 trafficking remains normal [12–14]. Conversely, in insulin resistant muscle, sarcolemmal FAT/CD36, but not FABPpm, is upregulated. However, insulin-, and contraction-stimulated FAT/CD36, but not FABPpm, translocations are impaired (cf. [3]). Collectively, it appears that there may be (i) different exocytosis mechanisms for GLUT4 and FA transporters, and (ii) depending on the stimulus provided, different proteins may be required for the trafficking of GLUT4 and/or FA transporters to the PM. Such a system would provide for selective regulation of GLUT4 and/or FA transporter trafficking to the PM.

Our aim was to examine the effects of Munc18c on insulin-, and contraction-stimulated glucose and FA transport. We hypothesized that Munc18c differentially affects (a) insulin- and contraction-stimulated glucose and FA transport and (b) the translocation of GLUT4 and FA transporters to the PM, as well as (c) contraction-induced mitochondrial FA oxidation and (d) FAT/CD36 trafficking to mitochondria [15,16].

2. Materials and methods

2.1. Animals

Due to an embryonically lethal homozygous genotype, we used male, 8–10 week old heterozygous Munc18c$^{-/+}$ mice [11] (22.7 ± 3.0 g) bred on site with C57/BL6 wildtype (WT) mice (24.1 ± 3.1 g), and kept at 22 °C, 40% humidity, 12:12-h light–dark cycle, and given chow and water *ad libitum*. Experiments were performed on anesthetized mice (sodium pentobarbital 6 mg/100 g body wt ip; MTC Pharmaceuticals, Cambridge, ON, Canada) using principles of laboratory animal care (National Institutes of Health publication No. 85-23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm). The Munc18c$^{-/+}$ genotype was confirmed using standard DNA isolation and PCR methods (Extract-N-Amp, Sigma–Aldrich, St. Louis, MO, USA), as we have reported previously [11].

2.2. Experimental treatments

Fasted (12 h) WT and Munc18c$^{-/+}$ animals (N = 4–6/experiment) were designated as control, or treated with (a) insulin (Humulin, 1.0 U/kg body wt, ip, 15 min; Eli Lilly, Toronto, ON, Canada), or (b) muscle contraction (sciatic nerve stimulation: 3 × 5 min, 2 min rest between stimulations, 100 Hz/3 s, 5 V, train 200 ms, pulse 10 ms) [17,18]. Homogenates, giant sarcolemmal vesicles (GSV) and mitochondria were prepared from hindlimb muscles [17–19]. Intraperitoneal glucose (1.0 g/kg body wt) and insulin (1.0 U/kg body wt) tolerance tests were determined in separate animals. Tail vein glucose was determined using a glucose meter (Ascensia Elite XL, Bayer Inc., Toronto, ON, Canada).

2.3. Giant sarcolemmal vesicles and substrate transport

GSV from hindlimb muscles were used to determine FA and glucose transport and the presence of PM transport proteins, as described previously [18,19].

2.4. Mitochondrial isolation and palmitate oxidation

After muscle contraction, hindlimb muscles were harvested for isolation of subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria, for determination of FA oxidation, using standard procedures [15–17]. Mitochondrial recovery is 26% [20] and they are highly purified [21].

2.5. Western blotting

Protein levels were measured in GSV, muscle homogenate, and mitochondria using Western blotting [17,18]. Antibodies against Munc18c and syntaxin4 (each 1:1000) were generated in-house [11]. Antibodies against GLUT4 (1:4000) (Millipore, Temecula, CA), FAT/CD36, FATP1, FATP4 (each 1:500) (Santa Cruz Biotechnology, Santa Cruz, CA, USA [22]), AMPK and Akt2, and phosphorylated AMPKα Thr^{172}, Akt Thr^{308}, and Akt Ser^{473} (each 1:1000) (Cell Signaling (Danvers, MA, USA). FABPpm (1:30000, J. Calles-Escandon, Wake Forest University) and MCT1 (1:3000, H. Hatta, University of Tokyo) were gifts. Secondary antibodies, (Santa Cruz Biotechnology), were used as follows: Munc18c, syntaxin4, GLUT4, FABPpm and MCT1 (1:3000 anti-rabbit), FAT/CD36 (1:5000 anti-mouse), FATP1 (1:5000 anti-rabbit), FATP4 (1:5000 anti-goat), and all total and phosphorylated Akt and AMPK (1:1000 anti-rabbit). Blots were analyzed with the ChemiGenius2 Bioimaging and GeneTools software (SynGene, Cambridge, UK) [17,18]. Ponceau-S staining was used to ensure protein loading, as well as COXIV (Invitrogen, Burlington, ON; 1:30000 dilution) for mitochondria.

2.6. Statistics

Data were analyzed using analysis of variance and Fisher's LSD post hoc test when appropriate. All data are reported as mean ± sem.

3. Results

A 50% reduction in Munc18c protein (Fig. 1), along with a comparable reduction in mRNA (data not shown, [11,23]) occurred in skeletal muscle of Munc18c$^{-/+}$ animals. Syntaxin4 and transport proteins (GLUT4, FAT/CD36, FABPpm, FATP1, FATP4) remained unaltered (Fig. 1A). Basal blood glucose concentrations were comparable in mice (WT (6.1 mM ± 1.1; Munc18c$^{-/+}$ 6.3 ± 1.0 mM). Munc18c$^{-/+}$ mice exhibited insulin resistance and glucose intolerance (Fig. 1 B and C).

3.1. Knockdown of Munc18c does not affect insulin-, or contraction-mediated signal transduction

Insulin increased the phosphorylation of Akt-Ser^{473} (+150%) and Akt-Thr^{308} (+220%) comparably in WT and Munc18c$^{-/+}$ mice (Fig. 2A and B), as reported previously [11]. For unknown reasons, muscle contraction did not alter Akt phosphorylation (Fig. 2) as observed elsewhere [24], possibly owing to differences in the contractile stimulus used (Fig. 2). Total Akt2 was comparable in WT and Munc18c$^{-/+}$ mice (Fig. 2C).

Muscle contraction, not insulin, increased AMPKα Thr^{172} phosphorylation (+400%) (Fig. 2D). Total AMPKα was comparable in WT and Munc18c$^{-/+}$ mice (Fig. 2E).

3.2. Knockdown of Munc18c reduces insulin-, but not contraction-mediated glucose transport and GLUT4 translocation

Basal glucose transport and PM GLUT4 were comparable between WT and Munc18c$^{-/+}$ mice (Fig. 3), whereas PM Munc18c was reduced 50% (Fig. 4A). In WT mice, insulin increased glucose transport (+133%) and PM GLUT4 (+60%) (Fig. 3), whereas in Munc18c$^{-/+}$ mice, insulin-stimulated glucose transport and GLUT4