FEBS Letters 583 (2009) 2294–2300

journal homepage: www.FEBSLetters.org

Additive effects of insulin and muscle contraction on fatty acid transport and fatty acid transporters, FAT/CD36, FABPpm, FATP1, 4 and 6

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ARTICLE INFO

Article history:
Received 8 April 2009
Revised 29 May 2009
Accepted 8 June 2009
Available online 13 June 2009

Edited by Dietmar J. Manstein

Keywords:
Giant vesicle
Electrical stimulation
Plasma membrane
Muscle
Membrane translocation
GLUT4

ABSTRACT

Insulin and muscle contraction increase fatty acid transport into muscle by inducing the translocation of FAT/CD36. We examined (a) whether these effects are additive, and (b) whether other fatty acid transporters (FABPpm, FATP1, FATP4, and FATP6) are also induced to translocate. Insulin and muscle contraction increased glucose transport and plasmalemmal GLUT4 independently and additively (positive control). Palmitate transport was also stimulated independently and additively by insulin and by muscle contraction. Insulin and muscle contraction increased plasmalemmal FAT/CD36, FABPpm, FATP1, and FATP4, but not FATP6. Only FAT/CD36 and FATP1 were stimulated in an additive manner by insulin and by muscle contraction.
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1. Introduction

In recent years it has been shown that fatty acids are transported into many tissues via a protein-mediated mechanism (for review see [1]). A number of fatty acid transporters have been identified, many of which are co-expressed in the same tissue. Two key fatty acid transporters are fatty acid translocase (FAT/CD36) and plasma membrane associated fatty acid-binding protein (FABPpm), each of which has been shown to stimulate fatty acid transport [1–3]. Chronically increased fatty acid transport rates has been linked with a permanent relocation of FAT/CD36 to the plasma membrane in type 2 diabetes (for review see [1]) and diet-induced insulin resistance [4].

Acute regulation of protein-mediated fatty acid transport can also occur. In skeletal muscle, contraction and insulin induce the translocation of FAT/CD36 and FABPpm from an intracellular depot(s) to the plasma membrane (for review see [1,5]). Tissue specificity appears to occur, as in heart insulin induces the translocation of FAT/CD36 but not FABPpm [1]. Even less is known regarding the translocation of a family of fatty acid transport proteins (FATP1-6), although their involvement in the regulation of fatty acid transport has been shown [6]. FATPs are expressed in a tissue-specific manner [7,8], and in muscle tissues FATP1, 4 and 6 are co-expressed [7,9]. FATP1-null mice are resistant to diet-induced insulin resistance [10]. Although FATP1 appears to be insulin sensitive in muscle [11], it is not known whether insulin and/or muscle contraction induce the translocation of FATP1, 4, or 6.

Metabolic challenges with insulin and muscle contraction/exercise have revealed that skeletal muscle glucose transport can be stimulated independently by insulin and by muscle contraction/exercise, and additively when these stimuli are combined [12], as these stimuli induce GLUT4 translocation to the sarcolemma from insulin- and contraction-sensitive intracellular depots. In this manner the uptake and utilization of glucose is elegantly controlled, either to re-establish euglycemia after a meal or to increase substrate provision when demand for this substrate is increased, such as during exercise.

As with glucose, fatty acids are also cleared from the circulation either after a meal [13] or when demand for this substrate is increased with exercise [14]. Yet, it is not known (a) whether an increased rate of uptake of fatty acids primarily requires an increase in plasmalemmal FAT/CD36 or whether other fatty acid transporters are also involved, and (b) whether muscle contraction and insulin stimulate fatty acid transport and transporters in an additive manner. Therefore, in the present studies we have examined

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doi:10.1016/j.febslet.2009.06.020

the effects of muscle contraction, insulin, and the combined effects of muscle contraction and insulin on (i) the rates of fatty acid transport into giant vesicles prepared from skeletal muscle and (ii) on the appearance of fatty acid transporters (FAT/CD36, FABPpm, FATP1, FATP4 and FATP6) at the plasma membrane in response to these stimuli. For reference purposes we also examined the effects of these stimuli on insulin- and contraction-stimulated glucose transport and sarcolemmal GLUT4.

2. Materials and methods

2.1. Animals

We used 8–12 week old, overnight fasted male mice C57BL/6J. They were bred on site, and housed in controlled temperature and humidity conditions on a 12:12-h light–dark cycle. Animals were provided with standard laboratory chow and water ad libitum. Rates of glucose and fatty acid transport as well as plasmalemmal fatty acid transporters were examined in giant vesicles prepared from hindlimb muscles of overnight fasted mice. There were four experimental groups: control, insulin stimulation, muscle contraction, or combined insulin and muscle contraction. Experiments were approved by the committee on animal care at the University of Guelph and were performed with anesthetized mice (sodium pentobarbital (6 mg/100 g body wt ip; MTC Pharmaceuticals, Cambridge, ON, Canada).

2.2. Experimental treatments

For treatments other than control (no treatment), animals were either (a) injected with insulin (Humulin, 1.0 U/kg body wt, i.p. (Eli Lilly, Toronto, ON, Canada)), (b) muscles were induced to contract (sciatic nerve stimulation; train delivery 100 Hz/3 s at 5 V, train duration 200 ms, pulse duration 10 ms), or (c) muscles were exposed simultaneously to the insulin and muscle contraction treatments. After 15 min, hindlimb muscles were harvested. Tail vein glucose concentrations were also measured (glucose monitor: Ascensia Elite XL, Bayer Inc., Toronto, ON, Canada).

2.3. Giant sarcolemmal vesicles and substrate transport

Giant sarcolemmal vesicles were prepared from hindlimb muscles immediately after harvest, as we have described previously in detail elsewhere [15] and were used to determine substrate transport rates and plasmalemmal proteins in the same preparation. Another rapid separation procedure [16] was used to visualize the presence of fatty acid transporters at the sarcolemma and intracellular depots. Protein concentrations were determined by the bicinchoninic acid (BCA) assay, and vesicles were used immediately for fatty acid and glucose transport assays or stored at −80 °C for Western blotting analyses.

Palmitate transport rates (15 s) into giant sarcolemmal vesicles were determined in separate experiments, as described previously [15,17–19], using unlabeled palmitate (14 μM) and radiolabeled 0.3 μCi [^{3}H]-palmitate (GE Healthcare, Baie D'urfe, QC, Canada) in a 0.1% bovine serum albumin solution. Glucose transport was determined using unlabeled D-glucose (5 mM) and radiolabeled 0.3μCi [^{3}H] D-glucose (GE Healthcare).

2.4. Western blotting

Transport proteins were detected on the plasma membrane of the giant vesicles, and on subfractionated sarcolemmal and endosomal preparations by Western blotting as we have described previously [17]. Standard procedures (SDS–PAGE) were used to separate proteins and transfer them onto PVDF membranes. Thereafter, membranes were blocked in 7.5% BSA for 1 h, and then probed overnight at 4 °C with the antibodies against FAT/CD36 (sc13572), FATP1 (sc25541), and FATP4 (sc5834) (each 1:500 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), FATP6 (1:1000, gift, Dr. A. Stahl, Palo Alto Medical Foundation Research Institute, Palo Alto, CA), FABPpm (1:30 000 dilution) was a gift from Dr. J. Calles-Escandon (Wake Forest University), GLUT4 (1:4000 dilution; Millipore, Temecula, CA, USA) and monocarboxylate transporter 1 (MCT1, 1:3000; gift from Dr. H. Hatta, University of Tokyo). Blots were detected using enhanced chemiluminescence (Perkin Elmer Life Science, Boston, MA) and quantified by densitometric analyses (ChemiGenius2 Bioimaging, SynGene, Cambridge, U.K.). All blots were stained with Ponceau-S to ensure equal loading of protein.

2.5. Statistics

Data were analyzed using analysis of variance and Fisher's LSD post-hoc test when appropriate. All data are reported as means ± S.E.M.

3. Results

Blood glucose was monitored to ensure a reduction with insulin treatments. Basal glucose concentrations were 6.48 ± 0.09 mM. This was not reduced by muscle contraction, but was reduced by the insulin (−70%) and the insulin + contraction (−69%) treatments.

3.1. Glucose transport and GLUT4

Muscle contraction or insulin increased the rates of glucose transport 2.5- and 3.6-fold respectively (Fig. 1A). When the two stimuli were combined, glucose transport was increased 5-fold (Fig. 1A). Plasma membrane GLUT4 was upregulated when muscles were stimulated with contraction (+76%), insulin (+154%) and by the combined contraction and insulin stimulation (+277%) (Fig. 1B). As a positive control plasma membrane MCT1, which is not translocated [20,21], was not altered during these experiments (Fig. 1C). These experiments confirm that the insulin and muscle contraction induced the well-known independent and additive effects on glucose transport and GLUT4 translocation.

3.2. Effects of insulin and muscle contraction on fatty acid transport and fatty acid transporters

Palmitate transport was increased by muscle contraction (1.7-fold), by insulin (2.9-fold), and by the combination of contraction and insulin (3.5-fold) (Fig. 2A). Concomitantly, plasma membrane fatty acid transporters were increased with muscle contraction (FAT/CD36 +55%; FABPpm +62%; FATP1 +61%; FATP4 +66%), insulin (FAT/CD36 +78%; FABPpm +61%; FATP1 +84%; FATP4 +60%), and with combined muscle contraction and insulin (FAT/CD36 +179%; FABPpm +85%; FATP1 +125%; FATP4 +89%) (Fig. 2B–E). Subfractionation of muscles showed that these foregoing transporters were induced to translocate from intracellular depots to the plasma membrane (Fig. 3). Plasma membrane content of FATP6 was not altered by insulin or muscle contraction (Fig. 2F), and therefore their combined effect on plasmalemmal FATP6 was not examined.

3.3. Additive effects of insulin and muscle contraction on glucose and fatty acid transport, GLUT4 and fatty acid transporters

For glucose transport the combined effects of muscle contraction and insulin were quite comparable, as with the combined stimuli (insulin + contraction) glucose transport was almost identi-

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