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Research Article

Reversible stress-induced lipid body formation in fast twitch rat myofibers

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ABSTRACT

We analyzed the existence of lipid bodies (LBs) in the fast twitch rat flexor digitorum brevis (FDB) myofibers and found that these structures were scarce. However, isolation procedure of the myofibers, heath shock, viral infection or the glycosylation inhibitor tunicamycin induced formation of the LBs, which were stationary structures flanking Z lines. We next infected FDB myofibers with recombinant Semliki Forest virus expressing caveolin 3-yellow fluorescent protein (cav3-YFP) since this chimeric protein was targeted to the LBs facilitating their further analysis. Photobleaching experiments showed that the LBs recovered cav 3-YFP extremely slowly, indicating that they were not continuous with the endoplasmic/sarcoplasmic reticulum. We found, however, that cav3-YFP could move from the LBs to the sarcolemma and this phenomenon was sensitive to Brefeldin A, suggesting that the chimeric protein could be returned from the LBs to the endoplasmic reticulum.

Introduction

Lipid bodies (LBs) consist of a neutral lipid core containing triglycerides, diacylglycerides and cholesterol esters. They are coated with a monolayer of phospholipid and cholesterol that includes the PAT (perilipilin, ADRP, TIP47) proteins and a large set of other proteins. LBs are abundant in adipocytes but they are present in all other cell types, too, including skeletal muscle. They were formerly regarded as inert lipid storage bodies, however, owing to the functional protein set in their coat they are nowadays considered dynamic organelles of lipid metabolism and intracellular lipid trafficking. The coating proteins regulate the storage and mobilization of the lipids and are subject to disturbances that result in lipid degradation intermediates that are cell signaling molecules. This is especially relevant in skeletal muscle cells and therefore the LBs in skeletal muscle have recently gained interest in research of obesity and type 2 diabetes. Accordingly, the amount of LBs in skeletal muscle cells is linked with insulin resistance [1,2]. This lipid-induced insulin resistance is mediated by insulin desensitizing lipid intermediates such as diacylglycerol, ceramide and protein kinase C [3–5].

It has been shown that LBs are present in type I myofibers but practically absent in type IIb myofibers while type IIa myofibers represent intermediate expression pattern. The LB-specific coat

Abbreviations: ADRP, adipocyte differentiation related protein; BFA, brefeldin A; cav3-YFP, caveolin 3-yellow fluorescent protein; ER/SR, endoplasmic/sarcoplasmic reticulum; FDB, flexor digitorum brevis; HRP, horse radish peroxidase; LB, lipid body; PDI, protein disulfide isomerase; SFV, Semliki Forest virus; SREBP, sterol regulatory element-binding protein; TIP47, 47 kDa tail interacting protein; VSV, vesicular stomatitis virus

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proteins OXPAT and ADRP follow this distribution pattern [6]. It has been shown previously that physical exercise decreases the intramuscular lipid content in exercising muscle but increases it in non-exercising muscle [7], indicating that the amount of LBs can be regulated. Interestingly, it has been observed that elevated fatty acid levels induce the formation of LBs in a wide range of mononucleated cells [8]. Furthermore, ER stress in several cell types plays an important role to induce LB formation that is associated with metabolic diseases [9]. Here we found that the fast twitch type IIa flexor digitorum brevis (FDB) myofibers [10] contain scarcely LBs but these structures could be induced under culture conditions. Accordingly, massive amounts of LBs could be induced by the myofiber isolation procedure, by viral infection, by subjecting the myofibers to 41 °C, or by tunicamycin treatment. Many of these factors induce ER stress that seems to cause LB formation in the FDB myofibers. Compatible with the situation in BHK and Vero cells in which caveolin 3-green fluorescent protein (cav3-GFP) accumulated in LBs under certain conditions [11], we found here that caveolin 3-yellow fluorescent protein (cav3-YFP) accumulated in the nascent LBs in the FDB myofibers. Upon photobleaching of a given LB the YFP fluorescence did not recover indicating that these structures were not continuous with the ER. Our results also suggest that cav3-YFP could be transferred from the LBs back to the endoplasmic/sarcoplasmic reticulum (ER/SR).

Materials and methods

Isolation of FDB myofibers

Myofibers were isolated from the FDB muscle of three months old female Sprague Dawley rat footpads by collagenase digestion as described earlier [12,13]. The isolated myofibers were grown on Matrigel-coated (Beckton Dickinson) plastic (NUNC) or glass-bottomed (Ibidi) cell culture dishes in Dulbecco's MEM supplemented with 5% horse serum in an atmosphere of 5% CO_2 at 37 °C. With the exception of data in Table 1, myofibers were used for experiments after 24 h cultivation period.

LB staining

LBs were visualized by using oil red O and a standard protocol [14] or by using the LD540 fluorescent dye that was synthesized as described by Spandl et al. [15]. Frozen sections (10 μm) and isolated myofibers were fixed with 2.5% paraformaldehyde for 20 min and then the dye was applied for 5 min at 0.1 μg/ml concentration. Live myofibers were stained at 0.5 μg/ml concentration for 5 min. The fraction of LB-positive myofibers in sections or on each dish was examined with Zeiss AxioScope A1 epifluorescence microscope with a 20x plan neo fluor objective by scanning through the entire section or dish. In the quantitations mean and standard deviation (SD) are indicated.

Table 1 – Isolation of myofibers induces LB formation. Myofibers were isolated from rat FDB and plated on Matrigel-coated dishes and cultivated for the indicated time periods. The myofibers were then fixed with paraformaldehyde and LBs were visualized with the LD540 dye. The mean percentages of LB-containing myofibers are indicated together with the SD of 3 determinations.

Time from plating, h	% myofibers containing LBs
2	68.7±8.9
10	46.2±7.7
24	22.3±8.5
48	10.3±4.9
72	2.6±0.8

Recombinant virus and viral infections

cDNA encoding mouse cav3-YFP was excised from the plasmid cav3-YFP-N1 [8] that was kindly provided by Dr. Robert Parton (University of Queensland, Brisbane). Excision was with KpNI/NotI digestion. The cDNA was blunted and ligated to pSFV1 [16] that was opened with SmaI. Recombinant viruses expressing cav3-YFP were prepared as described by Olkkonen et al. [17]. Multiplicities of infection of the virus stocks used varied between 10^7–10^8 infective particles/ml as evaluated on BHK cell monolayers. For infections, the virus stocks were diluted 1:5 with the culture medium and allowed to adsorb for 16 h at 37 °C. Infections with wild type Semliki Forest virus (SFV) and vesicular stomatitis virus (VSV) were performed as previously described [18]. The doses of infections were adjusted to obtain over 90% of the myofibers infected and the infections were allowed to propagate for 20 h.

In vivo electroporation

Animal treatments were according to the national animal welfare laws that meet EU Directive 2010/63. Cav3-YFP-N1 plasmid (5 μg/μl) was injected in a volume of 15 μl into FDB muscle of an anesthetized rat. Electroporation was then applied at 200 V/cm by using custom-made electrodes. The animal was sacrificed 1 h later and the myofibers were isolated and cultured on Matrigel-coated dishes.

Cholesterol treatment

Myofibers were infected with pSFV-cav3-YFP for 24 h and after that the medium was replaced with culture medium containing 150 μg/ml water soluble cholesterol (Sigma). After a 24 h incubation period at 37 °C the myofibers were fixed with 2.5% paraformaldehyde for 20 min. The YFP fluorescence was then observed with Zeiss LSM 510 confocal microscope using 100x objective.

Confocal microscopy

Cells were fixed with 2.5% paraformaldehyde in PBS for 20 min at room temperature. ADRP was visualized using specific antibodies raised in guinea pig and DyLight 594-conjugated anti-guinea pig IgG (Progen). Sterol regulatory element-binding protein (SREBP) was visualized using monoclonal antibody against amino acids 301–407 (BD Pharmingen) and Alexa 488 conjugated IgG (Life Technologies) as secondary antibodies. Incubations with anti-SREBP1-antibody were performed at room temperature for 4 h. Nuclei were visualized with TO-PRO-3 iodide (Life Technologies). YFP fluorescence in fixed cells was observed on plastic dishes covered with a coverslip and in live cells on glass-bottom dishes