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Genetic obesity alters recruitment of TANK-binding kinase 1 and AKT into hypothalamic lipid rafts domains

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ABSTRACT

Lipid rafts (LRs) are membrane subdomains enriched in cholesterol, glycosphingolipids and sphingolipids containing saturated fatty acid. Signaling proteins become concentrated in these microdomains mainly by saturated fatty acid modification, thus facilitating formation of protein complexes and activation of specific signaling pathways. High intake of saturated fatty acids promotes inflammation and insulin resistance, in part by disrupting insulin signaling pathway. Here we investigate whether lipid-induced toxicity in obesity correlates with altered composition of insulin signaling proteins in LRs in the brain. Our results showed that insulin receptor (IR) is highly concentrated in LRs fraction in comparison with soluble or postsynaptic density (PSD) fractions. Analysis of LRs domains from hippocampus of obese mouse showed a significant decrease of IR and its downstream signaling protein AKT, while in the PSD fraction we detected partial decrease of AKT and no changes in the IR concentration. No changes were shown in the soluble extract. In hypothalamus, genetic obesity also decreases interaction of AKT, but we did not detect changes in the IR distribution. However, in this structure genetic obesity increases recruitment of the IR negative regulator TANK-binding kinase 1 (TBK1) into LRs and PSD fraction. No changes of AKT, IR and TBK1 were found in soluble fractions of obese in comparison with lean mice. *In vitro* studies showed that incubation with saturated palmitic acid but not with unsaturated docosahexaenoic acid (DHA) or palmitoleic acid decreases association of IR and AKT and increases TBK1 recruitment into LRs and PSD domains, emulating what happens in the obese mice. TBK1 recruitment to insoluble domains correlates with decreases of IR tyrosine phosphorylation and ser473 AKT phosphorylation, markers of insulin resistance. These data support the hypothesis that hyperlipidemia associated with genetic obesity alters targeting of TBK1 and insulin signaling proteins into insoluble LRs domains.

1. Introduction

The hypothalamus is a region of the central nervous system (CNS) involved in the control of energy balance through the integration of peripheral metabolic signals at the level of orexigenic and anorexigenic neurons (Sandoval et al., 2008). Disruption of signaling integration in these areas might result in insulin resistance states, including obesity and diabetes.

In the context of obesity, failure of adipose tissue expandability and function cause leakage of fatty acids and ectopic accumulation in peripheral metabolically relevant organs including the hypothalamus resulting in cellular toxicity and body metabolic complications, a process called lipotoxicity (Carobbio et al., 2011). The quality of the lipid species and their derivatives has relevant implications on metabolic diseases. Accumulation of ceramides, diacylglycerols or saturated fatty acids is deleterious compared to triglycerides, malonyl-CoA, long-chain fatty acyl-CoA or polyunsaturated lipids in liver, adipose tissue, muscle and hypothalamus (Lopez et al., 2008; Medina-Gomez et al., 2005, 2007; Yetukuri et al., 2007). This evidence indicates that alterations in hypothalamic lipid metabolism during obesity may play a primary/secondary pathogenic role in the context of the metabolic disturbance associated with the Metabolic Syndrome.

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Changes in the lipid profile might modulate lipid rafts (LRs) microdomains composition and signaling pathway integration into these domains (Yaqoob, 2009). LRs are small (10–200 nm), heterogeneous, highly dynamic, cholesterol and sphingolipid enriched membrane domains composed of tightly packed saturated fatty acids (Yaqoob, 2009). Signaling proteins with affinity for rafts become concentrated in these regions coordinating the activation of specific signaling pathways (Simons et al., 1998b). Saturated lipid modifications such as palmitoylation, myristoylation or glycosylphosphatidylinositol (GPI)-anchors provide the affinity of proteins for LRs domains (Delint-Ramirez et al., 2011; Levental et al., 2010). In addition, saturated fatty acids promote formation and protein recruitment into these domains, whereas polyunsaturated fatty acids (PUFA) supplementation destabilize LRs by disrupting protein palmitoylation (Chapkin et al., 2000; Kim et al., 2008; McMurray et al., 2000; Schley et al., 2007). Thus, quality of lipids species is also important in modulating rafts functionality and might have important nutritional/therapeutic implications in metabolic related disorders.

Altered integrity of LRs in several organs has been involved in diverse aspects of the insulin resistance state leading to diabetes. TNF-induced insulin resistance in adipocytes results in accumulation of the GM3 ganglioside, decreasing the number of insulin receptors within the rafts-related domains (Kabayama et al., 2007). In addition, obesity induced by high fat diet disrupts the Cbl/CAP/TC10 raft insulin-signaling cascade in visceral adipose tissue in mice (Jun et al., 2008; Luan et al., 2009). Furthermore, protein acylation is also important in the coordination of food intake; for example, octanoylation of ghrelin by the Ghrelin O-Acyltransferase is required to perform its cellular functions in the hypothalamus (Kojima and Kangawa, 2005; Yang et al., 2008). Palmitoylation is also required for proper function of alpha subunit of G protein receptor (Resh, 2006), its mutant form has been associated with obesity, insulin resistance and diabetes (Chen et al., 2009). In summary, protein acylation and LRs modulation might represent an integrative platform to coordinate signaling pathways important for body energy homeostasis in peripheral tissues and brain.

In the present study, we sought to investigate whether metabolic abnormalities associated to lipid-induced toxicity during obesity induce altered targeting of insulin signaling proteins to LRs in the hypothalamus.

2. Materials and methods

2.1. Antibodies

The following antibodies were used insulin receptor (beta subunit), flotillin, actin, postsynaptic density 95, AKT, Src, TANK-binding kinase 1 (TBK1), N-methyl-D-aspartate receptor (NR1, NR2A and NR2B subunits).

2.2. Animal models

All the experiments were performed using wild-type and ob/ob 6 month old mice. Animals were handled according to the NIH guide for the care and use of laboratory animals (NIH Publications No. 80-23, revised in 1996), with approval of the local Animal Care Committee. All efforts were made in order to minimize the number of animals used and their suffering. Animals were housed in a temperature-controlled room with a 12-h light/dark cycle. Food and water were available *ad libitum*.

2.3. Lipid raft isolation

Lipid rafts were prepared from cell extracts as described previously with some modifications (Delint-Ramirez et al., 2010, 2011; Delint-Ramírez et al., 2008). Cells were homogenized in raft lysis buffer (150 mM NaCl, 25 mM Tris–HCl pH 7.5, containing 50 mM NaF, 10 mM NaP2O7, 1 mM Sodium orthovanadate, complete protease inhibitor cocktail (Roche, Mannheim, Germany) and 0.5% Triton X-100). Following homogenization by sonication (for 5 s at 1500 Hz on ice) the sample was centrifuged for 10 min at 1500× *g* and protein concentration determined by BCA assay. The supernatant (3 mg of protein in 500 μL per sample) was incubated for 30 min at 4 °C and centrifuged for 20 min at 16,000× *g* at 4 °C, to separate a Triton-soluble extract and the insoluble pellet. The pellet was resuspended in 0.4 mL of lysis buffer and mixed with 2 M sucrose (0.8 mL), overlaid with 1 M (1.6 mL) and 0.2 M (0.8 mL) sucrose and centrifuged for 15 h at 200,000× *g* at 4 °C. After centrifugation, five 0.8 mL fractions were collected from the top to the bottom of the gradient. Lipid rafts are enriched in the fraction 2, as previously reported. The pellet was resuspended in 100 μL of lysis buffer. Protein concentration was measured by BCA assay. The proteins in the gradient fractions were pelleted by centrifugation at 200,000× *g* for 45 min following dilution with 25 mM Tris–HCL, 150 mM NaCl. For the indicated experiments fractions 2 and 3 (lipid rafts) and fractions 4 and 5 (high density) were pooled and pelleted together. The pellets were resuspended in 100 μL of lysis buffer and protein concentration determined by BCA assay.

2.4. Neuronal culture

Cortical mouse neurons were cultured as described (Papadia et al., 2005). Cortex was dissected from embryonic E17 pups (1 cortex per culture) in Neurobasal A medium supplemented with B27 (Invitrogen), 1% rat serum, and 1 mm glutamine (Invitrogen) and cultured for 10–14 days as described (Papadia et al., 2005). Fatty acid stimulation was made in trophically deprived medium using palmitic acid (60 μM in NaOH 0.1 N), palmitoleic acid (60 μM), DHA (60 μM in DMSO) or DMSO for 12 h. After stimulation, cells were washed with cold buffer phosphate saline (PBS) and homogenized with raft lysis buffer as described.

2.5. mHypoA CLU472 hypothalamic cell line

Cell line was grown in growth medium (1x DMEM with 10% fetal bovine serum (FBS), 25 mM glucose and 1% penicillin/streptomycin) and maintained at 37 °C with 5% CO2. The cells were split when they reached 70–90% confluent (plate ratio of 1:4) using trypsinization at 37 °C for 1–5 min, followed by washing/resuspension in growth medium. Fatty acid stimulation was made in trophically deprived medium using palmitic acid (60 μM in ethanol), palmitoleic acid (60 μM), docosahexaenoic acid, DHA (60 μM in DMSO), or DMSO for 12 h or 24 h. After stimulation, cells were washed with cold buffer phosphate saline (PBS) and homogenized with raft lysis buffer as described.

2.6. Insoluble and soluble domains isolation

Insoluble and soluble domains were prepared following the published articles (Ma et al., 2003; Nunez-Jaramillo et al., 2008) with some modifications. Cells were homogenized in raft lysis buffer (150 mM NaCl, 25 mM Tris–HCl pH 7.5, containing 50 mM NaF, 10 mM NaP2O7, 1 mM Sodium orthovanadate, complete protease inhibitor cocktail (Roche) and 0.5% Triton X-100). Following homogenization by sonication (for 5 s at 1500 Hz on ice) the sample was centrifuged for 10 min at 1500× *g*. The supernatant was incubated for 30 min at 4 °C and centrifuged for 20 min at 16,000× *g* at 4 °C, to separate a Triton-soluble extract and the insoluble pellet. Soluble and insoluble extract proteins were determined by BCA assay.