Experimental Neurology 306 (2018) 34–44

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research paper

The cytoskeleton in 'couch potato-ism': Insights from a murine model of impaired actin dynamics

Karen Gertz[a,*,1], Ria Uhlemann[a,1], Anna Foryst-Ludwig[b,c], Ruben Marquina Barrientos[a], Kai Kappert[d], Christa Thöne-Reineke[e], Pierre Djoufack[f], Clemens Kirschbaum[g], Klaus B. Fink[f], Andreas Heinz[h], Ulrich Kintscher[b,c], Matthias Endres[a,c,i,1], Golo Kronenberg[h,j,1]

[a] Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health, Klinik und Hochschulambulanz für Neurologie und Centrum für Schlaganfallforschung Berlin (CSB), 10117 Berlin, Germany
[b] Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health, Institut für Pharmakologie, Center for Cardiovascular Research (CCR), 10115 Berlin, Germany
[c] DZHK (German Center for Cardiovascular Research), partner site Berlin, 10115 Berlin, Germany
[d] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Center for Cardiovascular Research/CCR, Institut für Laboratoriumsmedizin, Klinische Chemie und Pathobiochemie, 10115 Berlin, Germany
[e] Institut für Tierschutz, Tierverhalten und Versuchstierkunde, Freie Universität Berlin, 14163 Berlin, Germany
[f] Institut für Pharmakologie, Universitätsklinikum Bonn, 53113 Bonn, Germany
[g] Institut für Biologische Psychologie, Technische Universität Dresden, 01062 Dresden, Germany
[h] Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health, Klinik für Psychiatrie und Psychotherapie, 10117 Berlin, Germany
[i] Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), 10117 Berlin, Germany
[j] Klinik und Poliklinik für Psychiatrie und Psychotherapie, Universitätsmedizin Rostock, 18147 Rostock, Germany

ARTICLE INFO

Keywords:
actin
corticosterone
depression
hypoactivity
metabolic syndrome
obesity

ABSTRACT

Evidence for a critical pathophysiological role of aberrant cytoskeletal dynamics is being uncovered in a growing number of neuropsychiatric syndromes. A sedentary lifestyle as well as overt psychopathology is prevalent in patients with the metabolic syndrome. Using mice deficient in gelsolin (*Gsn*$^{-/-}$), a crucial actin-severing protein, we here investigated reduced actin turnover as a potential common driver of metabolic disturbances, sedentary behavior, and an anxious/depressive phenotype. Gelsolin deficiency resulted in reduced lifespan. As compared to wildtype controls, *Gsn*$^{-/-}$ mice (~ 9 weeks) fed a high-fat diet (HFD) over a span of 12 weeks showed increased body weight gain, fat mass, hepatic steatosis, and adipocyte hypertrophy as well as a significantly reduced respiratory quotient. Moreover, increased rigidity of the actin cytoskeleton in mice on HFD induced mRNA expression of *Acc1*, *Acc2*, *Fasn*, and *Lipe*, key genes involved in fatty acid metabolism in the liver. Glucose tolerance and insulin sensitivity were worsened in *Gsn*$^{-/-}$ HFD relative to *Gsn*$^{+/+}$ HFD mice. Hypertension in *Gsn*$^{-/-}$ mice was associated with reduced endothelial NO synthase (eNOS) mRNA expression and reduced eNOS protein trafficking to the plasma membrane. Furthermore, acetylcholine-induced cGMP production and relaxation of aortic rings were impaired by actin filament stabilization. *Gsn*$^{-/-}$ mice on HFD displayed reduced corticosterone concentrations and reduced energy expenditure as compared to *Gsn*$^{+/+}$ HFD mice. Moreover, *Gsn*$^{-/-}$ HFD mice displayed an overall pattern of hypoactive and anxious/depressive-like behavior. In aggregate, our results demonstrate that impaired actin filament dynamics promote the development of key behavioral and physiological aspects of the metabolic syndrome.

1. Introduction

In eukaryotes, the actin molecule represents the most abundant intracellular protein making up approximately 1 - 5 percent by weight of total protein in nonmuscle cells (Lodish, 2013). The actin cytoskeleton is essential to numerous cellular functions. Not only does it regulate cell shape and movement, but it also contributes to the transduction of environmental signals and provides tracks for intracellular

* Corresponding author at: Klinik für Neurologie, Charité Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany.
E-mail address: karen.gertz@charite.de (K. Gertz).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.expneurol.2018.04.004
Received 7 July 2017; Received in revised form 19 March 2018; Accepted 6 April 2018
Available online 21 April 2018
0014-4886/

transport (Pollard and Cooper, 2009). Actin is eminently sensitive to oxidative stress, which may result in oxidation of cysteine residues, intermolecular disulfides, and decreased overall cytoskeletal plasticity (Bencsath et al., 1996; Dalle-Donne et al., 2001). In turn, reduced turnover of filamentous (F)-actin may cause cellular dysfunction and, ultimately, cell death (Franklin-Tong and Gourlay, 2008; Leadsham et al., 2010; Wang and Gundersen, 1984). Exciting new research is increasingly pointing to a crucial role of impaired actin dynamics in a vast array of neuropsychiatric disorders and aging-related pathologies. For example, altered actin turnover has recently been implicated in mediating ethanol preference (Kronenberg et al., 2015; Ojelade et al., 2015). Moreover, the Alzheimer's-related proteins tau and Aβ have been shown to promote actin filament stabilization (Frandemiche et al., 2014; Fulga et al., 2007; Henriques et al., 2010). Conversely, increased actin dynamics have been linked with longevity (Gourlay et al., 2004). Finally, the complex interplay between diet and the actin cytoskeleton is gradually emerging as another important research topic. An energy-restricted diet has been shown to impact the expression of cytoskeletal genes alongside the expression of genes linked to inflammation, the extracellular matrix, and angiogenesis in murine adipose tissue (Higami et al., 2006). Interestingly, studies in genetically modified mice also suggest a role for the actin cytoskeleton in mediating the effects of high-fat feeding. KRAS-induced actin-interacting protein (KRAP) associates with F-actin. Notably, KRAP-deficient mice are resistant to HFD-induced obesity (Fujimoto et al., 2009). More recently, Murakami and co-workers demonstrated that adipocyte adhesion molecule (ACAM) modulates the dynamics of cell adhesion and actin polymerization in lipocytes and that ACAM-transgenic mice fed a high-fat, high-sucrose diet, are protected from obesity (Murakami et al., 2016).

Gelsolin is among the most potent actin filament severing proteins (Sun et al., 1999). Gelsolin-deficient ($Gsn^{-/-}$) mice therefore constitute an attractive model to study the effects of increased rigidity of the actin meshwork on a systems biology level (Witke et al., 1995). In the present report, we aim to delineate the wide-ranging effects of pathological actin filament stabilization on blood pressure, glucose metabolism, fat metabolism, and body weight *in vivo*. In particular, we focus on the interplay between impaired actin dynamics in $Gsn^{-/-}$ mice and high-fat feeding and conduct a detailed neurobehavioral assessment including measures of spontaneous activity, anxiety, hedonia, learning, and depressive-like symptoms.

2. Material and Methods

2.1. Animals and treatments

All experimental procedures were approved by the respective official committees and carried out in accordance with the Animal Welfare Act, the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the ARRIVE guidelines (Kilkenny et al., 2010). Mice lacking gelsolin ($Gsn^{-/-}$) have been described in detail previously (Azuma et al., 1998; Endres et al., 1999; Witke et al., 1995). Because the gelsolin-null state is not viable in either pure C57Bl/6 or BALB/C backgrounds, these animals have been crossed in a mixed 129/SV × C57BL/6 background for more than 10 generations. Unless otherwise indicated, male mice were used. Animals were housed in a temperature-controlled facility with a 12-hour light/dark cycle. Randomly assigned groups of $Gsn^{-/-}$ and $Gsn^{+/+}$ mice were fed ad libitum with a high fat diet (HFD; 60% kcal from fat, D12492 Research Diets, BROGAARDEN, Gentofte, Denmark) or a low fat diet (LFD; 10% kcal from fat, D12450B Research Diets, BROGAARDEN, Gentofte, Denmark) for 12 weeks. Body weight and food intake were recorded over the course of the experiments. Food intake on either experimental diet did not differ between $Gsn^{+/+}$ and $Gsn^{-/-}$ mice (not shown).

2.2. Assessment of body composition

Body composition was determined in awake mice by nuclear magnetic resonance (NMR; Echo Medical Systems, Houston TX, USA).

2.3. Calorimetric measurements

A custom-made 4-cage calorimetry system (LabMaster; TSE Systems GmbH, Bad Homburg, Germany) was used. Briefly, the instrument consists of a combination of feeding and drinking sensors for automated online measurement. The calorimetry system is an open-circuit system that determines O2 consumption, CO2 production, energy expenditure and respiratory quotient (*RQ*). A photobeam-based activity monitoring system records the movements of the experimental animals, including rearing and climbing movements, in every cage. All parameters are measured continuously. After appropriate adaptation, mice were studied in the calorimetry system cages for 24 h (Foryst-Ludwig et al., 2008).

2.4. Glucose tolerance test (GTT) and insulin tolerance test (ITT)

Mice were fasted overnight before testing. For the GTT, glucose (Glucose 20%; B. Braun) was injected intraperitoneally at a dose of 1g/kg body weight. For the ITT, insulin (INSUMAN Rapid 40 IU/ml, Sanofi-Aventis, Frankfurt am Main, Germany) was injected at a dose of 0.5 units/kg body weight. Blood from the tail vein was used to measure blood glucose levels 0, 15, 30, 90, 120 and 150 min after injection with a blood glucose monitoring system (FreeStyle Precision, Abbott, Wiesbaden, Germany).

2.5. Measurement of blood pressure and heart rate

Blood pressure measurements were essentially performed as described previously (Tiemann et al., 2003). Briefly, mice were anesthetized with 1.0% isoflurane in 69% N_2O and 30% O_2. Core temperature was maintained at 36.5 ± 0.5°C. The left common carotid artery was catheterized under a dissecting microscope (Carl Zeiss, Jena, Germany). In a few mice, telemetric recordings were performed for confirmation (Custodis et al., 2011).

2.6. Acetylcholine-induced relaxation of aortic rings

The aortas of female mice (P120) were dissected and transferred to Petri dishes containing Krebs' solution composed of (in mM): Glucose, 5.5; NaCl, 118.5; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2.5 (all from VWR International, Darmstadt, Germany). The adventitia and small vessels around the aorta were carefully removed under a dissecting microscope and the aorta cut into 2 mm rings, mounted onto two pieces of 40 μm tungsten wire submerged in Krebs' solution in a 4-channel multi-myograph system chamber (Model 610M, Myonic, Aarhus N, Denmark). The chamber was maintained at 37°C and aerated with 95% O_2/5% CO_2. Resting tension of aortic rings was adjusted over 30 min at 10 mN. The rings were then stimulated by changing the bathing solution to a depolarizing solution containing 125 mM KCl. Once the ring had reached maximal contraction, depolarizing solution was replaced by Krebs' solution and the rings were allowed to return to resting tension. In order to study maximal relaxation, increasing concentrations of acetylcholine or sodium nitroprusside were administrated after precontraction with 1 μM phenylephrine. Relaxation is expressed as percentage of phenylephrine-induced initial contraction.

2.7. NO-induced cGMP production

The aorta was transferred to a Petri dish containing Krebs' HEPES solution composed of (in mM): Glucose, 5.5; NaCl, 143.3; HEPES, 15;