Journal of Steroid Biochemistry & Molecular Biology 168 (2017) 60–70

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Diabetes alters myelin lipid profile in rat cerebral cortex: Protective effects of dihydroprogesterone

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ARTICLE INFO

Article history:
Received 17 December 2016
Received in revised form 31 January 2017
Accepted 2 February 2017
Available online 3 February 2017

Keywords:
Neuroactive steroids
Phosphatidylcholines
Phosphatidylethanolamines
Plasmalogens
Cholesterol
Myelin basic protein

ABSTRACT

Due to the emerging association of diabetes with several psychiatric and neurodegenerative events, the evaluation of the effects of this pathology on the brain function has now a high priority in biomedical research. In particular, the effects of diabetes on myelin compartment have been poorly taken into consideration. To this purpose, we performed a deep lipidomic analysis of cortical myelin in the streptozotocin-induced diabetic rat model. In male rats three months of diabetes induced an extensive alterations in levels of phosphatidylcholines and phosphatidylethanolamines (the main species present in myelin membranes), plasmalogens as well as phosphatidylinositols and phosphatidylserines. In addition, the levels of cholesterol and myelin basic protein were also decreased. Because these lipids exert important functional and structural roles in the myelin compartment, our data indicate that cerebral cortex myelin is severely compromised in diabetic status. Treatment for one-month with a metabolite of progesterone, dihydroprogesterone, restored the lipid and protein myelin profiles to the levels observed in non-diabetic animals. These data suggest the potential of therapeutic efficacy of DHP to restore myelin in the diabetic brain.

1. Introduction

Diabetes mellitus causes functional and structural changes in the nervous system, such as peripheral neuropathy [1,2], and encephalopathy [3,4]. In particular, the interest in the effect of this pathology on the brain is growing due to its association with cognitive deficits and increased risk of dementia, stroke, cerebrovascular and Alzheimer disease, as well as psychiatric disorders [5,6]. Among the different effects so far observed in the central nervous system (i.e., neurophysiological and structural changes in white and gray matter), the impact of diabetes on myelin compartment has been also investigated. Indeed, observations obtained in an experimental model of type 1 diabetes, such as the rat-raised diabetic by injection with streptozotocin (STZ), suggest that the gene expression of some myelin proteins was significantly decreased by STZ injection [7,8]. Myelin proteins exert an important role in the maintenance of the multilamellar structure of myelin [9,10]. In agreement, alterations of myelin membranes in central nervous system (CNS) areas (e.g., split myelin sheaths and myelin balloons) have been also reported [11]. On the other hand, myelin is highly enriched in lipids, such as cholesterol, glycosphingolipids and plasmalogens [10,12]. Indeed, in myelin the overall ratio of lipids to proteins is very high [13]. Myelin lipids contribute to myelin biogenesis, axon-glia communication and in long-term maintenance of myelin [12]. However, despite their important role, the effect of diabetes on myelin lipids of CNS has been poorly considered. In addition, a further background to explore the effect of diabetes on these myelin components could be also provided by our recent data obtained in the sciatic nerve myelin of STZ-treated rats. Indeed, lipidomic analysis in peripheral nervous system (PNS) myelin indicated that diabetes induces a

Abbreviations: Acc1, acetyl coenzyme A carboxylase alpha; Cer, ceramide; Elovl5 and Elovl6, fatty acid elongase 5 and 6; Fads1 and Fads2, fatty acid desaturase 1 and 2; DHA, docosahexaenoic acid; DHP, dihydroprogesterone; Fas, fatty acid synthase; GalCer, C17 mono-sulfo galactosyl(β) ceramidea; GCer, glucosyl/galactosyl-ceramide; GC, glucosyl (β) C12 ceramide; LysoPA, lysophosphatidic acid; LysoPC, lysophosphatidylcholine; LysoPI, lysophosphatidylinositol; MBP, myelin basic protein; PA, phosphatidic acid; PC, phosphatidylcholine; PCaa, phosphatidylcholine acyl-acyl; PCae, phosphatidylcholine acyl-alkyl; PE, phosphatidylethanolamine; PEaa, phosphatidylethanolamine acyl-acyl; PEae, phosphatidylethanolamine acyl-alkyl; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLP, myelin proteolipid protein; PS, phosphatidylserines; PUFA, polyunsaturated fatty acids; Scd-1, stearoyl coenzyme A desaturase 1; SM, sphingomyelin; Srebp-1c, sterol regulatory element binding protein-1c; STZ, streptozotocin; Sulfs, sulfatides.

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http://dx.doi.org/10.1016/j.jsbmb.2017.02.002
0960-0760/

decrease of cholesterol as well as of monounsaturated fatty acids associated with an increase in polyunsaturated fatty acids (PUFA) [14–16]. Interestingly, we have also demonstrated that the 5alpha-reduced metabolite of progesterone, dihydroprogesterone (DHP) was able to counteract these effects [14]. In particular, this neuroactive steroid was able to reduce accumulation of myelin-associated saturated fatty acids and to promote desaturation [14]. These findings are in line with the protective effects exerted by neuroactive steroids, like for instance DHP, on other functional and molecular parameters of diabetic peripheral neuropathy [17] suggesting for the first time that myelin lipid compartment, at least in PNS, may be considered a target for the action of these molecules [14].

In different experimental models of neurodegeneration, DHP as well as its precursor (i.e., progesterone) have been also demonstrated to be protective [18,19]. In this context, it is important to highlight that, as mentioned above, DHP is the 5alpha-reduced metabolite of progesterone and that both neuroactive steroids are able to interact with the progesterone receptor [20]. 5alpha-reductase enzyme is expressed in the nervous system [21] and it represents an important step in the mechanism of action of progesterone [20]. The protective effects mediated by progesterone receptor also include those on myelin compartment, affecting myelin protein expression and density of progenitor and mature oligodendrocytes [22,23]. However, whether activation of progesterone receptor by DHP treatment may affect myelin lipids in CNS areas, similarly to what observed in PNS myelin, remain to be evaluated.

To this aim, the effects of diabetes and the treatment with DHP have been assessed in the whole cerebral cortex of STZ-treated rats analyzing the myelin lipid profile by flow injection analysis tandem mass spectrometry (FIA-MS/MS), liquid chromatography tandem mass spectrometry (LC–MS/MS) and gas chromatography-mass spectrometry (GC–MS).

2. Methods

2.1. Reagents

All HPLC solvents were MS grade (Carlo Erba Reagents, Italia), 5α-pregnane-3,20-dione (dihydroprogesterone, DHP), 5α-cholestane, streptozotocin (STZ) and fatty acids internal standards: 13C-palmitic acid (C16:0) and 13C-linoleic acid (C18:2) were purchased from Sigma-Aldrich (Milano, Italy). Phospholipid standards: C13:0 lysophosphatidylcholines (LysoPC); C25:0 phosphatidylcholines (PC); C12:0 sphingomyelin (SM); 12:0–13:0 phosphatidylserine (PS); 12:0-13:0 phosphatidylinositol (PI); 12:0–13:0 phosphatidylglycerol (PG); 12:0–13:0 phosphatidic acid (PA); 12:0–13:0 phosphatidylethanolamine (PE); C12 ceramide (Cer); glucosyl (β) C12 ceramide (GC); lactosyl (β) C12 ceramide (LacCer); C17 monosulfo galactosyl(β) ceramide (D18:1/17:0; GalCer) were purchased from Avanti Polar Lipids (USA).

2.2. Animals

Procedures including animals and their care were performed in agreement with institutional guidelines that are in compliance with national (D.L. No. 26, March 4, 2014, G.U. No. 61 March 14, 2014) and international laws and policies (EEC Council Directive 2010/63, September 22, 2010: Guide for the Care and Use of Laboratory Animals, United States National Research Council, 2011). Upon arrival, after a week to acclimate the animals to our vivarium, we randomized the 2-month-old male Sprague-Dawley rats (Crl:CD BR, Charles River, Lecco, Italy) in blind to have five control (CTRL; non diabetic) rats, and ten receiving STZ. In particular, the induction of diabetes was made in ten rats by a single intraperitoneal injection of STZ, 65 mg/kg (Sigma-Aldrich, Milano, Italy) in 0.09 M citrate buffer pH 4.8 and the remaining five control (non diabetic) animals were injected only with the same volume of buffer citrate, as previously described [24,25]. Glycemia was measured two days post-induction and only rats with blood glucose concentrations >300 mg/dl were included in the study. Two months after the induction of diabetes, animals were randomized into two groups: STZ treated with vehicle (sesame oil) and STZ treated with DHP. Specifically, the randomization was done on the STZ-treated rats that were divided in two groups (control sesame oil treated rats and those receiving DHP) keeping the weight and the glycemia comparable and not statistically different between the two groups. Both groups were compared with a healthy non-diabetic group (CTRL) treated with vehicle (sesame oil). All animals were treated every other day for a month with a subcutaneous injection of vehicle or DHP (3.3 mg/kg body weight). The treatment schedule applied was the same previously demonstrated by us to exert neuroprotective effects in diabetic experimental model [14,26]. Special care was taken to minimize animal suffering and to reduce the number of animals used, which is the minimum required for statistical accuracy. Moreover, to minimize the number of animals used in the experimentation we applied a power analysis. By means of G power software we used F-test ANOVA analysis (repeated measures) by setting: the effect size (f) at 0.75 (obtained with the same software on the basis of previous experiments), the alpha error probability at 0.05, the power of the test ≥80% and we obtained 5 animals per group.

2.3. Gene expression profile in cerebral cortex

RNA was extracted from whole cerebral cortex of control, STZ and STZ treated with DHP animals with a Nucleospin RNA II kit (Macherey-Nagel) and analyzed by a TaqMan qRT-PCR (quantitative real-time) run on a CFX384 real time system (Bio-Rad Laboratories). Samples were run in 384-well format in triplicate as multiplexed reactions and data were normalized with an internal control (36B4). The iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories) was used for the RNA amplification. Probe and primer sequences (Eurofins MWG-Operon) are available on request.

2.4. Free cholesterol quantification by gas chromatography-mass spectrometry (GC–MS)

Myelin was purified from cerebral cortex as previously described [27]. Methanolic extracts of myelin-enriched fractions were used for free cholesterol quantification. First, 60 μl of the methanolic extracts were derivatized with a mixture of bis-trimethylsilyltrifluoroacetamide (BSTFA):pyridine (4:1 v/v) for 30 min at 60 °C, and then analyzed with a gas chromatograph-mass spectrometer (GC–MS, Varian Saturn 2100). The electron impact ionization mode was used for the MS analysis. GC–MS analysis was executed as described: 1 μl sample was injected in split less mode (inlet was kept at 270 °C with the helium flow at 1.0 ml/min) at the initial 180 °C for 1 min, then ramped at 50 °C/min to 240 °C and at 5 °C/min to 300 °C. The ions selected for the quantification were at *m/z* 368 for cholesterol and *m/z* 357 for 5α-cholestane (IS) and calibration curve freshly prepared were used for the quantification.

2.5. Phospholipid and fatty acid profiles by flow injection analysis-tandem mass spectrometry (FIA-MS/MS) and liquid chromatography-tandem mass spectrometry (LC–MS/MS)

Quantification of total fatty acid contents was performed by LC–MS/MS as previously described [14–16]. For the quantification of