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ALTERATIONS IN PHOSPHOLIPIDOMIC PROFILE IN THE BRAIN OF MOUSE MODEL OF DEPRESSION INDUCED BY CHRONIC UNPREDICTABLE STRESS

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Abstract—Depression is a worldwide disability disease associated with high morbidity and has increased dramatically in the last few years. The differential diagnosis and the definition of an individualized therapy for depression are hampered by the absence of specific biomarkers. The aim of this study was to evaluate the phospholipidomic profile of the brain and myocardium in a mouse model of depression induced by chronic unpredictable stress (CUS). The lipidomic profile was evaluated by thin layer and liquid chromatography and mass spectrometry and lipid oxidation was estimated by FOX II assay. Antioxidant enzyme activity and the oxidized/reduced glutathione (GSH/GSSG) ratio were also evaluated. Results showed that chronic stress affects primarily the lipid profile of the brain, inducing an increase in lipid hydroperoxides, which was not detected in the myocardium. A significant decrease in phosphatidylinositol (PI) and in cardiolipin (CL) relative contents and also oxidation of CL and a significant increase of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were observed in the brain of mice after unpredictable chronic stress conditions. In the myocardium only an increase in PC content was observed. Nevertheless, both organs present a decreased GSH/GSSG ratio when compared to control groups, corroborating the occurrence of oxidative stress. The enzyme activities of catalase (CAT) and superoxide dismutase (SOD) were found to be decreased in the myocardium and increased in the brain, while glutathione reductase (GR) was decreased in the brain. Our results indicate that in a mouse model for studying depression induced by CUS, the modification of the expression of oxidative stress-related enzymes did not prevent lipid oxidation in organs, particularly in the brain. These observations suggest that depression has an impact on the brain lipidome and that further studies are needed to better understand lipids role in depression and to evaluate their potential as future biomarkers.

Key words: chronic stress, brain, phospholipids, mass spectrometry, lipidomic, oxidative stress.

INTRODUCTION

Depression is a common and disabling psychiatric disorder with a lifetime prevalence of about 20%. Estimations from the World Health Organization predict that by the year of 2030 depression will be the second leading cause of disease burden worldwide (Joels et al., 2004; WHO, 2008; Huynh et al., 2011). Although the etiology of the disease remains to be completely understood, chronic stress has been considered one of the main factors that predispose the individuals to the development of depression (Krishnan and Nestler, 2008). During chronic stress, the hyperactivation of the sympathetic nervous system and a deregulation of the hypothalamus pituitary adrenal gland axis occur, leading to the increase of stress hormones, such catecholamine's and glucocorticoids (Chrousos, 2009; Hill et al., 2012). Up regulation of the stress hormones may induce the overproduction of free radicals and thus oxidative stress has been associated with depression progress (Michel et al., 2012). Oxidative stress induces changes in lipid profiling in consequence of lipid peroxidation, which has been associated with several neurological diseases. Also, the majority of lipid peroxidation products have pro inflammatory effects (Cohen, 2000; Black, 2002; Ahmad et al., 2010; Wielgat et al., 2011). Although, the pathophysiological alterations that trigger the association between chronic stress and depression are not completely understood, it is known that this association is characterized

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Abbreviations: BHT, 2,6-di-tert-butyl-*p*-hydroxytoluene; BSA, bovine serum albumin; CAT, catalase; CL, cardiolipin; CUS, chronic unpredictable stress; DAG, sn-1,2-diacylglycerol; FST, forced swimming test; GR, glutathione reductase; GSH, oxidized glutathione; GSSG, reduced glutathione; HPLC–MS, high-performance liquid chromatography–mass spectrometry; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIPs, phosphorylated phosphoinositides; PS, phosphatidylserine; ROS, reactive oxygen species; SM, sphingomyelin; SOD, superoxide dismutase; TLC, thin layer chromatography.

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by an increase in cytokines/inflammation and oxidative stress with an increase in reactive oxygen species (Miller, 2010). Individuals with severe depression have shown inflammation, manifested by an increase of pro inflammatory cytokine levels, such as tumor necrosis factor (TNF-α), interleukins (IL-1 e IL-6), observed in peripheral blood and cerebral spinal fluid, as well as an increase of the acute phase proteins, chemokines and adhesion molecules in peripheral blood (Miller, 2010).

Although chronic stress has been related with an increase in lipid peroxidation (Liu et al., 1996), its effect on brain lipid profile has not been investigated. The study of the physiological processes that occur in the brain during depression, based on biochemical mechanisms associated to neuronal membrane modifications, reflect an increase in lipid peroxidation in every brain region (Flerov and Gerasimova, 2007). This increase in lipid oxidation destabilizes membranes and may cause cell death (Tyurin et al., 2009).

The chronic unpredictable stress (CUS) paradigm is a widely used rodent model of depression, originally described by Katz and Hersh (1981) and further developed by Willner et al. (1987), which emphasizes the role of stress in the etiology of depression. In this model, animals gradually develop a chronic depressive-like state due to sequential and unpredictable exposures to different stressors, over a sustained period of time. CUS induces long-lasting changes of behavioral and neurobiological parameters resembling dysfunctions observed in depressed patients (Willner, 1997, 2005; Hill et al., 2012).

The aim of this study is to describe the alterations in phospholipid profile in the brain and myocardium in a mouse model of depression induced by CUS. The presence of oxidative stress promoted by CUS was validated by the oxidized/reduced glutathione (GSH/GSSG) ratio and the observation of lipid peroxidation. Some key enzymes against oxidative stress were also measured. The lipid profile was evaluated using a lipidomic approach: The different phospholipid classes were initially separated by thin layer chromatography (TLC) and quantified by phosphorous assay. The total lipid extracts from the brain and the myocardium were then analyzed by high-performance liquid chromatography–mass spectrometry (HPLC–MS) and MS/MS.

EXPERIMENTAL PROCEDURES

Chemicals

The phosphatidylethanolamine (PE – 14:0/14:0), phosphatidylserine (PS – 14:0/14:0), phosphatidylcholine (PC – 14:0/14:0), phosphatidylinositol (PI – 16:0/16:0), phosphatidic acid (PA – 14:0/14:0), sphingomyelin (SM – d18:1/18:1), phosphatidylglycerol (PG – 14:0/14:0), lysophosphatdyl choline (LPC – 18:0), lysophosphatidylinositol (LPI – 18:0), lysophosphatidylethanolamine (LPE – 18:0), lysophosphatidic acid (LPA – 18:0) and cardiolipin (CL – 14:0/14:0/14:0/14:0) standards were purchased from Sigma–Aldrich (Madrid, Spain), triethylamine (Acros Organics, Geel, Belgium), chloroform (HPLC grades), methanol (HPLC grades, Fisher Scientific, Leics, UK), ethanol (Panreac, Barcelona, Spain), primuline (Sigma–Aldrich, St. Louis, MO, USA) were used without further purification. TLC silica gel 60 plates with concentration zone (2.5 × 20 cm) were purchased from Merck (Darmstadt, Germany).

Animals

Male, 9-week old C57/BL6 mice (Charles River, Barcelona) were individually housed under a 12-h light/dark cycle in a humidity/temperature-controlled room, with *ad libitum* access to a standard chow diet and water, except when food and water deprivation was specified by the stress protocol. Animals were allowed 5 days to acclimatize to the surroundings before each stress protocol. All experimental procedures were performed in accordance with the European Union Directive 86/609/EEC for the care and use of laboratory animals. All people working with animals have received appropriate education (FELASA course) as required by the Portuguese authorities. In addition, animals are housed in our licensed animal facility (International Animal Welfare Assurance number 520.000.000.2006). The present study and the animal experimentation described were included in a project approved and financed by the Portuguese Science Foundation. Center for Neuroscience and Cell Biology (CNC) animal experimentation board also approved the utilization of animals for this project (reference PTDC/SAU-FCF/108110/2008).

CUS protocol

The CUS protocol was performed as previously described by Willner et al. (1987)), with some modifications. Mice were exposed to different stressors that were applied once a day, during a period of 21 days, and in the following order: day 1 – exposure to the box with wet shavings (24 h); day 2 – pairing with another stressed mice (1 h); day 3 – cold bath (15 °C, 20 min); day 4 – enclosure in a tube (2 h); day 5 – foot shock (0.7 mA, 3 s, given intermittently during a total time of 5 min); day 6 – exposure to the apparatus of foot shock without the shocks (1 h); day 7 – light off in the light phase of the cycle and box without shavings (24 h); day 8 – inclined box (45°, 24 h); day 9 – deprivation of water and food (24 h); day 10 – access to the empty bottle (1 h); day 11 – exposure to the box with wet shavings (24 h); day 12 – pairing with another stressed mice (1 h); day 13 – cold bath (15 °C, 20 min); day 14 – enclosure in a tube (3 h); day 15 – foot shock (0.7 mA, 3 s, given intermittently during a total time of 5 min); day 16 – exposure to the apparatus of foot shock without the shocks (1 h); day 17 – deprivation of water and food (24 h); day 18 – access to the empty bottle (1 h); day 19 – enclosure in a tube (4 h); day 20 – light off in the light phase of the cycle and box without shavings (24 h); day 21 – inclined box (45°, 24 h). Animals were used for behavioral tests or sacrificed for tissue collection 24 h after the last stressor.

Forced swimming test (FST)

The FST was performed to evaluate depressive behavior of mice (Cryan and Mombereau, 2004; David and John, 2012). Mice were dropped individually into glass cylinders