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Qualitative and quantitative changes in phospholipids and proteins investigated by spectroscopic techniques in animal depression model

J. Depciuch [a,*], M. Sowa-Kucma [b], G. Nowak [b,c], M. Papp [b], P. Gruca [b], P. Misztak [b,c], M. Parlinska-Wojtan [a]

[a] Institute of Nuclear Physics Polish Academy of Sciences, PL-31-342 Krakow, Poland
[b] Institute of Pharmacology, Polish Academy of Science, Department of Neurobiology, Laboratory of Trace Elements Neurobiology, Smetna Street 12, 31-343 Krakow, Poland
[c] Department of Pharmacobiology, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

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ABSTRACT

Depression becomes nowadays a high mortality civilization disease with one of the major causes being chronic stress. Raman, Fourier Transform Infra Red (FTIR) and Ultraviolet-Visible (UV–vis) spectroscopies were used to determine the changes in the quantity and structure of phospholipids and proteins in the blood serum of rats subjected to chronic mild stress, which is a common animal depression model. Moreover, the efficiency of the imipramine treatment was evaluated. It was found that chronic mild stress not only damages the structure of the phospholipids and proteins, but also decreases their level in the blood serum. A 5 weeks imipramine treatment did increase slightly the quantity of proteins, leaving the damaged phospholipids unchanged. Structural information from phospholipids and proteins was obtained by UV–vis spectroscopy combined with the second derivative of the FTIR spectra. Indeed, the structure of proteins in blood serum of stressed rats was normalized after imipramine therapy, while the impaired structure of phospholipids remained unaffected. These findings strongly suggest that the depression factor, which is chronic mild stress, may induce permanent (irreversible) damages into the phospholipid structure identified as shortened carbon chains. This study shows a possible new application of spectroscopic techniques in the diagnosis and therapy monitoring of depression.

1. Introduction

Depression is a common mental disorder, characterized by: sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, tiredness and poor concentration. In its most severe form, depression can even lead to suicide. The World Health Organization (WHO) estimates that by 2030 depression will be the 2nd leading cause of mortality worldwide [1,2]. The diagnosis is based on psychological tests and no markers or other diagnostic tools are available to confirm or exclude depression. Moreover, the neurobiological bases of depression are poorly understood, but abnormalities in the functioning of neural proteins and peptides activity seem to be crucial for the development of this disease. Furthermore, chronic stress has been considered as one of the main risk factors predisposing the individuals to develop a depression [3]. The organism reaction to long-term stress consists in the hyperactivation of the sympathetic nervous system and the dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. Consequently, an increase of the corticotropin releasing hormone (CRH) occurs, followed by adrenocorticotropic hormone (ACTH) secretion. This increase of ACTH concentration leads to the glucocorticoids (mainly cortisol and corticosterone) release from adrenal cortex [4,5]. As an effect of cortisol release the level of such lipids as cholesterol and triglycerides increases, leading consequently to the decrease of phospholipids [6,7]. Maes et al. reported in 1999, the relationship between the severity of depression and the amount of lipids in the membranes of red blood cells [8]. Other studies on depression [9–12] have shown that the increase in concentration of the triglycerides and cholesterol fraction in plasma membranes is compensated by the reduction of phospholipids quantity, proposing that the deficit of phospholipids could be a marker for depression [13]. Changes in the concentration of these three lipids affect the liquidity of membranes, resulting in transport dysfunctions, as well as in malfunction of important physiological processes.

The hypothesis that cortisol release increases the level of cholesterol and triglycerides was verified experimentally on animal models and clinical observations [14]. To determine the factors of pathogenesis, symptoms and effects of depression, scientists often stimulate the development of this disease in animals considering them as a model. The chronic mild stress (CMS) model is probably the most valid animal model of depression, as the animals behave very similar to depressed humans [15]. This model has been reported to result in long lasting changes of behavioral, neurochemical, neuroimmune, and neuroendocrinological variables, including decreased intracranial self-stimulation, reflecting anhedonia that is reversed by chronic, but not acute

* Corresponding author.
E-mail address: joannadepciuch@gmail.com (J. Depciuch).

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antidepressant treatment [16,17]. Moreover, in laboratory animals early deprivation of parents causes hypercortisolism and abnormal behavior, imitating the course of depression in humans [18–20].

Recently, spectroscopic techniques, such as Raman, FTIR and UV–vis, are increasingly used in biomedical sciences [21]. Spectroscopy methods provide information about the chemical composition, as well as about qualitative and quantitative changes in the investigated samples. Raman and FTIR spectroscopy thus seem to be ideal methods for chemical analysis of blood serum components, including proteins and phospholipids [22–24]. These techniques are complementary and mutually supporting [25], because the formation of spectra is based on different physical principles. FTIR requires the changes of dipole moment, while Raman requires a change in polarisability during vibration [26]. Thus the simultaneous use of both spectroscopic techniques, allows acquiring full spectra containing also these peaks, which might be not visible only by one of these methods. UV–vis spectroscopy is a spectroscopic method that enables quantitative analysis of the chemical compounds in the tested substance [27]. High resolution, fast measurements, low cost and minimal material preparation are some of the many advantages of these spectroscopic methods.

The aim of the present study was to determine the qualitative and quantitative changes occurring in phospholipids and proteins present in the blood serum of rats, which were subjected to chronic mild stress. Furthermore, the efficiency of the imipramine treatment was evaluated. For these purposes, Raman, FTIR and UV–vis spectroscopy were used.

2. Methodology

2.1. Animal Experiments and Blood Collection

2.1.1. Animals and Housing

The studies were conducted on male Wistar rats (Charles River, Germany), which were brought into the laboratory one month before the start of the experiment. Apart from those described below (see *Chronic mild stress procedure*), the animals were singly housed under standard laboratory conditions with a natural light/dark cycle, a temperature of 22 ± 2 °C and the humidity of 50 ± 5%. Food and water were freely available throughout the experiment. All of the experimental procedures were performed with a minimization of animal suffering and as few animals as possible used, according to the National Institute of Health Animal Care and Use Committee guidelines. The experimental procedures were approved by the Ethic Committee of the Institute of Pharmacology Polish Academy of Science in Krakow.

2.2. Chronic Mild Stress Procedure

Chronic mild stress (CMS) procedure was performed as described previously [28]. After adaptation to the laboratory and the housing conditions, the animals were first trained to consume a 1% sucrose solution. The training consisted of nine 1 h baseline tests in which sucrose was presented in the home cage, followed by 14 h of food and water deprivation. The sucrose intake was quantified by weighing the bottles with sucrose solution at the end of the test. Subsequently, sucrose consumption was monitored under similar conditions, at weekly intervals, throughout the whole experiment. On the basis of their sucrose intake in the final baseline test, the animals were divided into two groups. One (Stress) group of animals was subjected to the chronic mild stress procedure for a period of seven consecutive weeks. Each week of the stress regime consisted of: two periods of food or water deprivation, two periods of 45° cage tilt, two periods of intermittent illumination (light on and off every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low intensity stroboscopic illumination (150 flashes/min) and three periods without stress. All of the stressors were of 10–14 h duration and were applied individually and continuously, day and night. The second (Control) group of animals was housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for the 14 h preceding each sucrose test, but otherwise food and water were freely available in the home cage. Detailed schedule of experimental procedure is presented in Fig.1.

2.2.1. Drug Administration

On the basis of their sucrose intakes followed by initial 2 weeks of stress, both the stressed and the control (unstressed) groups were divided further into matched subgroups (n = 8/each group), and during the subsequent 5 weeks they received once daily 0.9% aqueous sodium chloride solution (saline) or imipramine (IMI, 10 mg/kg). The compounds were administered intraperitoneally (*i.p.*) in a volume of 2 ml/kg of body weight around 10.00 a.m. The animals were subjected to stress throughout the entire period of treatment. The weekly sucrose tests were carried out in the 24 h following the last drug injection. After 5 weeks and 24 h following the last antidepressant (or saline) treatment, all the experiment on animals was terminated.

2.2.2. Collection and Processing of Blood Samples

24 h after the last doses of antidepressant, the rats were sacrificed by rapid decapitation. The trunk blood was collected into tubes without anti-coagulant and allowed to clot for 15–20 min. After that, all the blood samples were centrifuged for 30 min at 1800 rpm at 4 °C. The resulting supernatant (serum) was quickly pipetted into tubes and stored at −80 °C until the start of spectroscopic analysis.

2.2.3. Spectroscopic Techniques

Directly before the analysis, all the blood serum samples were thawed and subsequently measured by Raman, FTIR and UV–vis spectroscopies. The samples were vortexed for 20 s prior to the measurement and the blood serum was collected from the bottom of the tube. All the measurements for each of the eight rats per group were performed in triplicates according to the following procedure. For each group 8 samples were measured: taking always the same volume of blood serum. Subsequently, two more measurements of samples from 1 to 8 were performed. Thus 3 × 8 measurements = 24 spectra from each group were acquired. Next, the 3 spectra from each sample were averaged using OPUS software.

2.2.4. Raman Spectroscopy

The serum samples were measured using a SmartRaman DXR spectroscope (Thermo Scientific, USA). Each sample was placed in a special

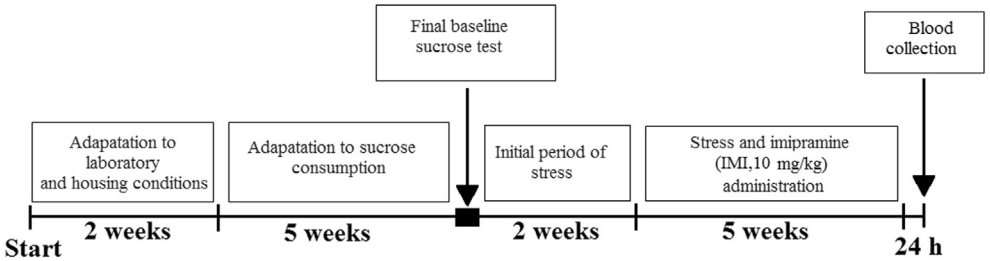

Fig. 1. Schematic schedule of the experimental procedure.