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Original research article

Olfactory bulbectomy-induced changes in phospholipids and protein profiles in the hippocampus and prefrontal cortex of rats. A preliminary study using a FTIR spectroscopy

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ABSTRACT

Background: Dysfunctions in neuronal proteins can lead to development of depression. Lipids determine the localization and function of proteins in the cell membrane and thereby regulate synaptic throughput in neurons. The aim of study was to examine changes in the phospholipid–protein balance in the olfactory bulbectomy (OB) model of depression in rats using a Fourier transform infra-red (FTIR) spectroscopy.

Methods: Sprague Dawley rats were subjected to the OB procedure and treated with amitriptyline (AMI, 10 mg/kg). The FTIR measurements were performed in the hippocampus and prefrontal cortex.

Results: The obtained results show a decrease in the phospholipids and protein fractions (as well as changes in their secondary structures) in both brain areas of bulbectomized rats. AMI treatment reduced phospholipids' and increased the proteins' brain level, yet did not affect the level of phospholipids or increase the proteins in OB rats. Second derivatives calculated from the FTIR spectra provided information that the proteins (but not phospholipids) in both structures of the OB_AMI rats were normalized after antidepressant treatment.

Conclusions: Our findings prove that the changes in the phospholipid-protein balance in the hippocampus and prefrontal cortex may be related to depressive disorders, thus leading to the development of adverse changes in the molecules necessary for the correct functioning of the brain. Furthermore, these results suggest a stability of the structure changes/damage of phospholipids in depression, which are present in brain tissues even after effective pharmacotherapy. This study also shows that an infrared spectroscopy can be applied for monitoring changes in mood disorders.

Introduction

Major depressive disorder (MDD, also known as unipolar depression, recurrent depression) is a mental disorder characterized by a permanent low mood that is accompanied by low self-esteem and by a loss of interest. According to the World Health Organization (WHO), MDD will the leading cause of global disability by the year 2030 [1,2]. Approximately 10% of patients with depression commit suicide [3]. The most common time of onset is between the ages of 20 and 30 years, with a later peak between 30 and 40 years [4–6]. Clinically, the diagnosis of MDD is based on the patient's self-reported experiences, supplemental information provided by family, and mental status examination. So far, there is no useful diagnostic laboratory test for major depression, which is the consequence of poor known mechanisms underlying this disorder [7,8].

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Although the etiology of depression is not fully understood, the disturbance of homeostasis in the brain has been considered one of main causes that predispose the individuals to the development of the disease [9]. The brain is one of the most lipid-rich organs and, therefore, it appears that the correct lipid turnover/metabolism is essential for its proper functioning [10,11]. There is evidence that lipids play an increasingly recognized role in neuronal function and their composition in the brain (including single brain regions, specific neuronal subjective subtypes and even neuronal subcompartments) substantially influences subjective perception, mood and emotional behavior [12–15]. A large number of lipids are especially located in the plasma membrane, where they constitute a physical barrier that segregates the inner and outer cellular environments. Membrane lipids are also involved in cell signaling and determine the localization or function of proteins within the membrane, plus in doing so regulate synaptic throughput [16]. One of the major lipid components of the brain membranes are phospholipids (glycerophospholipids and sphingolipids), which may be hydrolyzed into a second messenger that mediates acute responses [17]. Accordingly, changes in the (phosho)lipids profile could lead to different pathogenic processes in the brain. There are studies showing an imbalance in lipid metabolism in people suffering from seasonal affective disorder [18]. In addition, other researchers argue that in affective disorders a decreased amount of phospholipids and an increased cholesterol fraction is observed [19–22]. Therefore, it is presumed that phospholipids may be one of the markers of depression [17]. Changes in the amount of individual lipid fractions in the cell membrane negatively affect its liquidity. Consequently, structural changes and dysfunctions in the transport and membrane proteins are observed. To summarize, the phospholipid–protein imbalance can be an important factor in the development of affective disorders.

A large number of studies provide information concerning major depressive disorder being particularly associated with functional and structural changes within two brain regions: the hippocampus and frontal cortex. Analysis of the brain using the neuroimaging technique – positron emission tomography (PET) – showed, among other things, a reduced volume of these structures as a consequence of depression [23]. In turn, these alterations may be the result of a reduced or changed content of different proteins and lipids in the brain. PET is a very good method for imaging the anatomical modifications in the brain of depressed patients, but unfortunately gives no information about structural and biochemical changes accompanying the disease [24–26]. These details may be provided by using alternative methods, such as a FTIR spectroscopy. Nowadays, this technique, especially in combination with statistical tools, is becoming more widely used in the study of biological samples, as it is fast, simple, requires small quantities of the measured material, is economical, and does not destroy the sample. The energy of the electromagnetic wave in the infrared range is large enough to cause vibrations of the functional groups belonging to proteins and phospholipids, which is through a change in the dipole moment of bonding [27,28], and, therefore, the FTIR technique seems to be an ideal tool to analyze the alterations of phospholipid and protein profiles [29]. So far, there is no report on the use of a FTIR spectroscopy in studies of the pathomechanism of depression and for monitoring the progress of the pharmacotherapy.

The aim of our study was to investigate the balance between phospholipids and proteins in the rat hippocampus and prefrontal cortex. We used the bilateral olfactory model (OB), which is a widely accepted animal model of agitated depression [30] and, more importantly, agitated depression is a major risk factor for suicide [31].

Materials and methods

Animals and olfactory bulbectomy (OB) procedure

The experiments were performed on male Sprague-Dawley rats (Charles River Laboratories, Munich, Germany), initially weighing ~290 g (~8 weeks old). The animals were kept under controlled laboratory conditions (12-h light/dark cycle, constant temperature: 22 ± 2 °C and humidity: 55 ± 5%) with food and water freely available. During the experiment the body weight in each of the animal groups was constantly monitored (there were no significant differences between the average body weight in each experimental group at the beginning and the end of the experiment). All experimental procedures were conducted according to the guidelines of the National Institutes of Health Animal Care and Use Committee and after approval of the Ethics Committee of the Institute of Pharmacology, PAS in Krakow.

After 2 weeks' acclimation period, the animals were divided into two groups: OB and Sham rats, and then a bilateral olfactory bulbectomy was performed according to the method described by Pochwat et al. [32]. Briefly, the animals were anesthetized using an intramuscular (*im*) injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). As an anti-inflammatory drug, Metoxicam (0.05 mg/kg) was used 60 min before the bulbectomy and during two days following the OB procedure. An incision was made in the skin over the skull, and after exposing the skull, 2 mm diameter holes were drilled at the points 7 mm anterior to the bregma and 1.5 mm either side of the mid-line according to the Paxinos and Watson stereotaxic atlas [33]. Next, the olfactory bulbs were removed by suction and the holes were filled with haemostatic sponge in order to stop the bleeding before the skin was closed. Sham animals were treated the same way except the bulbs were left untouched. After surgery, the rats were kept four per cage (two sham + two bulbectomized). The animals were given 14 days to recover following surgery prior to the drugs' administration, and during this period they were handled daily by an experimenter to eliminate any aggressiveness that could otherwise arise. Two weeks after surgery, the OB and Sham rats were divided into the following subgroups: Sham_NaCl and OB_NaCl (treated with 0.9% sodium chloride) or Sham_Ami and OB_Ami (treated with amitryptyline at a dose of 10 mg/kg). These compounds were administered intraperitoneally (*ip*) chronically once daily for 14 days. The number of animals in each group was eight.

Brain tissues' collection and slides preparation

24 h after administration of the last dose of the amitriptyline or sodium chloride the animals were decapitated, and the complete brains were extracted and rinsed with cold brine saline. Subsequently, the brains were prepared in the process according to the method described by Miller and Dumas [34]. Thick coronal sections (4.5 μm) were cut using microtome (Leica Biosystems, Nussloch, Germany) before being placed on pre-prepared CaF_2 slides. The tissue slides were fixed on the ATR crystal and subjected to spectroscopic snaps measured in the infrared range. The sections of the hippocampus and prefrontal cortex were prepared from the same levels of the brain for each rat. The FTIR analysis was performed in 9 sections of each rat at levels −3.14 to −3.8 (in the hippocampus) and 3.5–2.6 (in the prefrontal cortex) in relation to the Bregma, according to the Paxinos and Watson stereotaxic coordinates [33]. Both the right and left frontal cortices and hippocampi were analyzed.

Infra-red spectroscopy measurement

The FTIR spectroscopy measurements were performed using the Vertex 70 (Bruker, Karlsruhe, Germany) spectrometer applying