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Maternal stress predicts altered biogenesis and the profile of mitochondrial proteins in the frontal cortex and hippocampus of adult offspring rats

Katarzyna Głombik[a], Aneta Stachowicz[b], Joanna Ślusarczyk[a], Ewa Trojan[a], Bogusława Budziszewska[a], Maciej Suski[b], Marta Kubera[a], Władysław Lasoń[a], Krzysztof Wędzony[c], Rafał Olszanecki[b], Agnieszka Basta-Kaim[a,*]

[a] Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Krakow, Poland
[b] Chair of Pharmacology, Jagiellonian University Medical College, 16 Grzegórzecka Street, 31-531 Krakow, Poland
[c] Laboratory of Pharmacology and Brain Biostructure, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Krakow, Poland

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ABSTRACT

Currently, much attention is focused on the influence of mitochondrial disturbances at the onset of depression. The goal of this study was to investigate the impact of prenatal stress (an animal model of depression) on the mitochondrial biogenesis proteins and mitoproteome profile in the frontal cortex and hippocampus of adult 3-month-old male rats following a prenatal stress procedure.

Our results show that rats that were exposed to prenatal stress stimuli displayed depression-like behaviors based on the sucrose preference and elevated plus maze tests. It has been found that the level of the PGC-1α protein was reduced in the frontal cortex and hippocampus of the adult offspring after the prenatal stress procedure. Moreover, in the frontal cortex, the level of the pro-apoptotic protein Bax was up-regulated. Two-dimensional electrophoresis coupled with mass spectrometry showed the statistically significant down-regulation of the mitochondrial ribosomal protein L12 (Mrpl12) and mitochondrial NADH dehydrogenase [ubiquinone] flavoprotein 2 (NDUFV2) as well as the up-regulation of the Tubulin Polymerization Promoting Proteins (Tppp/p25) in the frontal cortex. In contrast, in the hippocampus, the mitochondrial pyruvate dehydrogenase E1 component subunit beta, the voltage-dependent anion-selective channel protein 2 (VDAC2), and the GTP-binding nuclear protein RAN (RAN) were down-regulated and the expression of phosphatidylethanolamine-binding protein 1 (PEBP-1) was enhanced. These findings provide new evidence that stress during pregnancy may lead not only to behavioral deficits, but also to disturbances in the brain mitoproteome profile in adult rat offspring.

1. Introduction

Depression is a mental disorder of unknown origin. Several lines of evidence indicate that a number of key risk factors are involved in the pathogenesis of this disease, including disturbed monoamine transmission, immune system dysfunction, weaker action of neurotropic factors, hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis, and an increased influence of excitatory amino acids (Basta-Kaim et al., 2014a; Szczesny et al., 2014; Szymańska et al., 2009). Currently, much attention is paid to the potential influence of metabolic disturbances at the onset of this disease. In fact, epidemiological data indicate co-occurrence of depression with obesity, metabolic syndrome and type-2 diabetes (Bouwman et al., 2010; Penckofer et al., 2014). Clinical observations show that depression increases the risk for diabetes by approximately 60%, and people with diabetes are twice as likely to have depression compared to the general population (Anderson et al., 2001). Moreover, data found that patients suffering from both depression and diabetes are more likely to experience complications due to drug resistance, and their lifespan is shortened compared to those suffering from only one of these diseases (Katon et al., 2010; Lustman et al., 2000).

Recently, special interest has been paid to the energy metabolism disturbances observed in functional assays and magnetic resonance spectroscopy studies (Cavelier et al., 1995) in psychiatric disorders. It is well known that the brain exhibits high

* Corresponding author. Tel.: +48 12 662 32 73; fax: +48 12 637 45 00.
E-mail address: basta@if-pan.krakow.pl (A. Basta-Kaim).

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metabolic activity and increased oxygen consumption, and most of the energy used by the brain is in the form of ATP, which is generated by mitochondria. Brain mitochondria produce reactive oxygen species (ROS), buffer cytosolic calcium (Ca2++) in neurons, and regulate neurogenesis, cell survival and death (Chen and Chan, 2006). Moreover, the mitochondria in brain may play important roles in controlling the processes of neuroplasticity, including neural differentiation, neurite outgrowth, neurotransmitter release and dendritic remodeling (Mattson et al., 2008). Interestingly, it has been suggested that the mitochondria not only provide energy support for these processes, but also directly modulate their course (Cheng et al., 2010). Among others, an interesting aspect of mitochondrial function is its presumed interaction with epigenetic processes that are important in the pathogenesis of neuropsychiatric disorders, including depression. In fact, some studies demonstrated the direct mitochondrial metabolic effect on the availability of substrates, such as acetyl-CoA, ATP, and S-adenosylmethionine, for epigenetic regulation (Wallace and Fan, 2010). Together, these observations suggest that mitochondrial abnormalities may be a key factor in the formation of metabolic disturbances that subsequently lead to depression (Konradi et al., 2004).

In accord with these hypotheses, mitochondrial malfunctions in depression are demonstrated as changes in their morphology, decreases in mitochondrial respiration, increases in mitochondrial DNA (mtDNA) polymorphisms and the level of mtDNA mutations, down-regulation of the nuclear mRNAs and the proteins engaged in mitochondrial respiration. Notably, gene and protein expression studies showed the down-regulation of the enzymes and other factors involved in ATP generation and storage as well as an association of depression with the genes involved in mitochondrial function (Clay et al., 2012). Additionally, recent data demonstrated alterations in the number of mitochondria and their distribution in brain areas important in the pathogenesis of depression using electron microscopy (Cataldo et al., 2010). In general, diminished high-energy phosphates and decreased pH in the brain of depressive patients has been documented (Shao et al., 2008).

In agreement with the available data, the brain's mitochondrial pathology could be a consequence of genetic susceptibility or neurotransmitter system dysregulation, but may also result from environmental impacts, such as exposure to toxins, infection, or stress. Prenatal stress is one of the factors that impacts the embryonic development of the brain and alters the behavior of affected individuals later in life, indicating that early adverse life experiences can affect brain development and may be involved in the pathogenesis of depression. It is known that the rat prenatal stress procedure is one of the well-characterized animal models of depression. In this model, increased immobility time in the forced swim test, disturbances in sleep and cognitive functions, decreases in sexual behavior and abnormalities in the functioning of the immune and neuroendocrine systems have been observed (Basta-Kaim et al., 2014a,b; Budziszewska et al., 2010; Mairesse et al., 2012; Szczesny et al., 2014; Szymańska et al., 2009).

Based on these studies, it is plausible that prenatal stress may affect mitochondrial protein functions and the protein profile in specific brain areas involved in the pathogenesis of depression. In the present study, we investigated the effect of prenatal stress on the key factor of mitochondrial biogenesis – PGC-1α (Peroxisome proliferator-activated receptor-γ coactivator) as well as the pro- and anti- apoptotic proteins (Bax and Bcl-2, respectively) in the frontal cortex and hippocampus of adult 3-month-old offspring rats. Moreover, we applied two-dimensional electrophoresis coupled with mass spectrometry to investigate the influence of the prenatal stress procedure on the proteomic expression profile in the brains of prenatally stressed rats.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (200–250 g upon arrival) were obtained from Charles-River Laboratories (Germany) and were maintained under standard conditions (a room temperature of 23 °C, a 12/12 h light/dark cycle, with the lights on at 0800 h am), with food and water (2 bottles) available ad libitum. Two weeks after arrival, vaginal smears from the female rats were taken daily to determine the phase of the estrus cycle. On the proestrus day, females were placed with males for 12 h, and the presence of sperm in vaginal smears was checked the next morning. Pregnant females were randomly assigned to the control and stress groups ($n = 10$ in each group).

All experiments were performed to minimize the number of animals used and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Local Ethics Committee, Kraków, Poland (permit no. 699, 18.01.2010).

2.2. Stress procedure

The prenatal stress procedure was performed as we described previously (Basta-Kaim et al., 2014b; Budziszewska et al., 2010; Szymańska et al., 2009). Briefly, the pregnant females were subjected to three stress sessions daily, beginning at the 14th day of pregnancy until delivery. At 0900 h am, 1200 h pm and 500 h pm, the rats were placed in plastic cylinders (7 cm × 12 cm) and exposed to a bright light (150 W, 1800–2000 lx) for 45 min. Control pregnant females were left undisturbed in their home cages. Only offspring from litters containing 10–14 pups with a similar number of males and females were kept. Male offspring were selected from 21-day-old litters for the experiment. They were housed in groups of four animals per cage (1–2 animals from each litter) under standard conditions. The offspring of the control (unstressed) and stressed dams were first tested at 3 months of age for behavioral changes.

2.3. Sucrose preference test

Behavioral changes were assessed in the sucrose preference test, which was manifested as a reluctance to drink sweetened water (anhedonia). To conduct a two-bottle sucrose preference test, prenatally stressed ($n = 17$) and control ($n = 17$) male rats were trained to consume a 1% sucrose solution in three 1-hour-long sessions (from 0900 h am till 1000 h am) at 3-day-long intervals. In each training session, the positions of the water and sucrose bottles were switched to eliminate any placement preference effect. One week after the last adaptation training, the animals were deprived of food and water overnight, and the actual sucrose preference test was performed according to the method described by Willner (Willner et al., 1987).

The preference was calculated according to the following formula:

$$\%\,\text{Preference} = [(\text{Sucrose preference}/\text{Total fluid intake}) \times 100].$$

2.4. Elevated plus maze test

Anxiety behavior was assessed in both the control ($n = 17$) and the prenatally stressed ($n = 17$) rats. The elevated plus-maze test was performed according to the method previously described by Pellow (Pellow et al., 1985). The maze was elevated to a height of 50 cm above the floor and illuminated from beneath with only a dim light (15 W). To allow the animals to adapt to the experimental conditions, they were placed in the experimental room for 1 h before