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THE STUDY OF CHANGES FOR SEVERAL BIOCHEMICAL PARAMETERS OF OXIDATIVE STRESS IN ANIMAL MODELS FOR NEUROPSYCHIATRIC DISORDERS

Ph D THESIS ABSTRACT

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The current study aims to determine the significance of oxidative stress in neuropsychiatric pathology by assessing specific markers changes in Wistar white rats animal models representative of schizophrenia and autism, but also relevant for depression and anxiety disorders and Alzheimer's disease.

The glutathione peroxidase enzyme and the index of lipid peroxidation, the malondialdehyde, were the indicators evaluated in the first two experimental situations, in which animal models of schizophrenia by administration of methionine, and animal models of autism by prenatal exposure to different doses of valproic acid were deviced, considering the possible correlations between the used doses and their potential toxicological effects. Given the mentionings in literature on sage's therapeutic and antioxidant properties and the novelty on this issue, the effects induced by the administration of sage essential oil concerning the behavior in the two animal models mentioned above were also studied.

Given the the nucleus accumbens relevance for the positive symptoms of schizophrenia, in the third experimental situation, the changes occuring after lesioning the nucleus (through right unilateral injection of 6-hydroxydopamine) were assessed in animal models of rats, with reference to specific behavioral tests as well as to the key markers of oxidative stress. In these animal models there were also studied the effects of nicotine administration, due to the important role that smoking and nicotine itself exert in schizophrenia.

Oxidative stress in neuropsychiatric pathology

Oxidative stress is considered a biochemical event consisting in the upset of the oxidant/antioxidant balance in the sense of exceeding the antioxidant defense capacity. The extracellular or intracellular adverse events , represented by alterings of cellular physiological processes, may lead to increased synthesis of toxic molecules known as the reactive oxygen species (ROS).

Due to their high reactivity, free radicals generate harmful effects on the body, and are involved in numerous pathologies among which the most feared are cancer,

atherosclerosis, chronic inflammation and diabetes. It is well known that oxidative stress has an important role in the etiopathogenesis of several neuropsychiatric disorders, including schizophrenia, Parkinson's disease, Alzheimer's disease, anxiety and bipolar disorder.

Of all the tissues, the brain contains the highest percentage of unsaturated fats, which makes its cells the most vulnerable to free radicals attacks. Thus, some experiments concerning this matter, focused on the hippocampus, the seat of important cognitive mechanisms, that appears to be extremely vulnerable with aging, as oxidative stress seem to affect the long-term potentiation (LTP), known today as the most attractive model for information storage at the cellular level. In fact, some authors consider that the hippocampus would be the most vulnerable nervous area to free radical scavengers attacks.

Although the neurodegenerative disorders differ in many respects, they share a number of pathogenic aspects that relate to specific inappropriate protein aggregations (such as amyloid plaques in Alzheimer's disease or Lewy bodies in Parkinson's disease) , a series of genetic mutations and certain mitochondrial dysfunctions and biochemical changes leading to neuronal apoptosis; it’s worth mentioning all of this issues can be explained by a sustained oxidative stress.

6-hydroxydopamine has an important role in inducing oxidative stress, and actually it is widely used to induce experimental forms of Parkinson's disease in animals. The neurotoxic effects of 6 - hydroxydopamine involve the production of hydrogen peroxide and hydroxyl radicals, reducing the activity of GPx and the SOD enzymes, and increasing the level of malondialdehyde concentration the striatum.

Regarding oxidative stress in autism, comparative studies assessing the level of homocysteine and other biomarkers in autistic children, indicated higher levels of homocysteine, which is negatively correlated with GPx activity and suboptimal levels of vitamin B12. High levels of lipid peroxidation in autism defined by moderate or highly accentuated increases of the level of isoprostane also suggest intense oxidative stress.

Of major interest are the deficiences that occur in the glutathione metabolic pathways, concerning in particular alterings in the enzymes activity within the GSH redox system, such as the case for glutathione peroxidase, glutathione reductase,

glutathione S - transferase, suggesting cellular vulnerability resulting from the redox equilibrium imbalances.

Treatment and Research Methods

Treatment was performed by administering a single intraperitoneal injection to pregnant Wistar female rats on day 12.5 after conception, i.e. the moment of the neural tube enclosure during embryonic development.

Valproic acid, or VPA, is a well-known teratogen that induces autistic symptoms in the offspring of the pregnant (rats) females undergoing treatment. After initially trying administering a dose of 600 mg/kg body weight, reported to be the optimum dose for inducing autistic syptoms, as a result of the high percentage of death or miscarriage, the dosage was reduced to 500mg/kg body weight and there were also injected lower doses of 300 and 150 mg/kg VPA in order to assess the toxicology of the product. Prior to administration, the Sodium valproate was dissolved at a concentration of 250mg/ml in saline. The born pups were weaned on day 23 and assigned to cages in groups of three , taking into account VPA dose and sex. A total of 23 individuals were selected for the autistic group, divided into three subgroups depending on the VPA dose, comprising of 7, 9 and 7 individuals corresponding the doses of 500, 300 and 150 VPA mg/kg body weight. The control group comprised of an equal number of individuals of similar age. The experiments (the behavioral tests) were initiateded from the time the lots have reached an average weight of 150 g. At the end of the experiments, at 24 hours from the completion of the last behavioral test, after the animals were anesthetized and sacrificed, nervous tissue from the temporal lobe, the hippocampal area, was sampled.

In the second experiment concerning the induction of a model of schizophrenia, the method comprised in intraperitoneal administering of daily doses of methionine (250 mg/kg body weight), with two hours before the start of the training sessions. Prior to administration, the amount of methionine was dissolved at a concentration of 0.021mg/20ml in distilled water. The study lot included 10 male rats, the control group being the same control lot used in the first experiment, aforementioned. Upon completion of the last behavioral test, the animals were anesthetized and sacrificed, and nervous tissue was collected from the temporal area.

The treatment with sage essential oil following protocol was applied: two doses of sage oil were used, of 1% and 3 % concentrations, which were administered for five consecutive days before the start of behavioral testing, and then continued throughout the course thereof. There were selected approximately equal groups of rats (3 to 5 individuals) for each of the two oil concentrations, from the groups exposed to valproic acid in doses of 500 mg/kg and 300 mg/kg and from the group treated with methionine (six groups in total). Each of these groups were in turn placed in a special reduced ventilated room where they inhaled for 15 minutes the vapours from 0.2 ml sage oil (of the appropriate concentration), injected into a aromatherapy cup. After therapy, the rats were subjected to training sessions, using behavioral tests. Upon completion of behavioral testing, the animals were anesthetized and sacrificed, and nervous tissue from the temporal lobe was collected.

Characteristic for schizophrenia is the hyperactivity of the dopaminergic system in particular on the mesolimbic dopaminergic pathway connecting the midbrain ventral tegmental area to the nucleus accumbens. Thus, given the relevance of the area for the positive symptoms of the disorder, the relevance of this nucleus lesion on some specific behavioral tests as well as on the main markers of oxidative stress was studied. Moreover, given the important roles that smoking and nicotine itself exert in schizophrenia (it is traditionally considered that smoking in schizophrenics is a means of self-medication), the effects that nicotine administration has on these animal models were also evaluated.

Specific right-unilateral lesions of the dopaminergic neurons located in the NAcc were produced with 6-OHDA (dose of 8µg/4µl), by stereotactic neurosurgical procedures under aseptic conditions and under anesthesia (sodium pentobarbital 45 mg/kg body weight). One week after testing, nicotine was injected again in single dose (0.3 mg/kg body weight, ip.) in the group with the lesioned NAcc, and behavioral tests were repeated. More specifically, half of the animals operated on the nucleus accumbens were injected with nicotine and half were not, as to comparatively observe the effect of nicotine administration. After 24 hours from the final behavioral testing, all animals in the experimental situations were anesthetized and sacrificed, and serum and brain tissue of the prefrontal and temporal areas were sampled.

All rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC).

The research methods have included methods for behavioral study: Y-maze test, Elevated plus maze test, social interaction test, labyrinth T, forced swimming test, the 8-arm radial maze and biochemical methods used for determining the activity of superoxide dismutase, of glutathione peroxidase by the DNTB method, for determining the lipid peroxide by the reaction of malondialdehyde, the quantity of soluble protein by the Bradford method and the dosage of the total antioxidant capacity by chemiluminescence technique.

The study of the effects of valproic acid (prenatal exposure) and methionine (by subcutaneous injection) in autism rat models respectively schizophrenia in rat models

Behavioral changes after prenatal exposure to valproic acid

The results following the behavioral tests confirm the validity of this animal model as autistic model; the lots prenatally exposed to high doses of valproic acid (500 and 300 mg/kg) showed clearly autistic behavioral patterns, repetitive behavior, decreased socialization, reduction of the social memory index value and impaired predilection for new experiences. Impaired affective emotional behavior was ascertained by the appearance of more frequent and prolonged depressive states, inhibition and anxiety as a result of prenatal exposure to valproic acid, suggesting the significance of this model for depression and anxiety disorders. Cognitive ability was clearly affected in particular for the higher doses of valproic acid, as impairings in acquisition memory, working memory and spatial memory were observed.

Behavioral changes caused by intraperitoneal administration of methionine

The daily intraperitoneal administration of methionine, 250 mg/kg body weight over a long period of time, generated more frequent and prolonged depressive and anxious states compared to the control group, in particular exacerbating states of anxiety when interacting with normal conspecific individuals during for sociability assessment tests; the subjects displaying strong social withdrawal, lack of interest for social novelty

and lack of motivation to explore the environment. The impairment of spatial memory and long term memory disturbances were observed, but no occurrence of repetitive behavior as in the VPA exposed rats. An interesting aspect was noted in regard to the short-term memory deficit that has progressed, becoming apparent after two weeks of dosing. These symptoms are characteristic for schizophrenic negative component.

Behavioral changes after treatment with different doses of sage essential oil in the groups prenatally exposed to valproic acid, respectively in the groups injected intraperitoneally with methionine

Aromatherapy with sage oil extract of 1% and 3% concentration on the groups prenatally exposed to valproic acid in doses of 500 mg/kg and 300 mg/kg, induced an improvement in regard to cognitive performances by improving short term memory index and clearly contributed to the amelioration of depressive states in the case of the group 500 mg/kg VPA, for both the oil concentrations used. The improvement of anxiety states was visible only at the concentration of 3% essential oil of sage, for the 300 mg/kg VPA lot.

The treatment with sage oil of the methionine intraperitoneally injected lots had less visible effects, did not influence the short term memory index, all the more as this index was not affected by the administration of methionine; the only significant effect manifested by the sage oil was reducing the depression index values for the concentration of 1%.

The modification of some biochemical parameters of oxidative stress following prenatal exposure to valproic acid, methionine administration respectively, and correlations between markers of oxidative stress and behavioral indices changes

The analyzed biochemical parameters for the two animal models described above were the specific activity of the enzyme glutathione peroxidase and malondialdehyde concentration from the temporal lobe, the hippocampal area. In both experimental cases significant decreases were observed in the activity of glutathione peroxidase and increases of the value of lipid peroxidation index by high levels of malondialdehyde, suggesting a high degree of oxidative stress in the temporal lobe.

Through statistical processing using Pearson correlations significant correlations, positive or negative, were found between the studied biochemical indicators and most

behavioral indicators, highlighting the importance of oxidative stress in the interested pathologies.

The effects of a 6-hydroxydopamine induced lesion in the nucleus accumbens and of nicotine administration after the 6-hydroxydopamine induced lesioning on memory and oxidative stress

Nucleus accumbens lesion by unilateral right injection of 6- hydroxydopamine (dose of 8μg/4μl) caused sensorial indifference to food. This would be explained by the essential role that the accumbens has in reward behavior, especially food reward.

Clearly, this behavior impaired the cognitive processes, as evidenced by the time needed to complete the 8-arm radial maze which was 300 seconds, the maximum time the the rat may remain in the maze.

With regard to the effects of nicotine, a cholinergic receptor specific agonist, the obtained results indicate a clear enhancer effect, when considering the time needed to complete the 8-arm radial maze. Also the operated group of animals that received nicotine recorded the fewest working memory errors (index of short-term memory), and reference memory (long term memory index) in the same test.

With respect to the biochemical tests following behavioral tests , it was found that 6-hydroxydopamine induced lesion to the nucleus accumbens causes an increase of the malondialdehyde concentration, and a decrease of the total antioxidative capacity, obvious signs of oxidative stress.

Regarding the administration of nicotine, it's evident prooxidant effect was demonstrated by increasings of the superoxide dismutase specific activity and total antioxidant capacity, and decreasings of the concentration of malondialdehyde. In addition, administration of nicotine resulted in doubling the level of protein in the brain compared to the 6-hydroxydopamine lesioned group.

It is therefore possible to assume there is a correlation between memory and oxidative stress processes, given that the administration of 6-hydroxydopamine at accumbal level generated cognitive impairments associated with increased oxidative stress, while administration of nicotine after one week, improved operated animals cognitive performance, while reducing the level of central oxidative stress.

Conclusions

Given the results of this study we can say that :

1. Valproic acid prenatal exposure to doses of 300 and 500 mg / kg in rats causes cognitive deficits of short-term and long-term memory and of spatial orientation, increases the states of anxiety and depression, favours developing of a strong repetitive behavior and reduces sociability and in some measure social memory, the treated groups exhibiting the autistic symptoms mentioned in the literature.
2. The triggered effects were proportional to the dose used, but there were exceptions e.g. the dose of 300 mg/kg valproic acid induces a higher level of depression, as well as lower social recognition compared to the dose of 500 mg/kg valproic acid.
3. Prenatal exposure to the dose of 150 mg/kg valproic acid also induces behavioral changes, but not as pronounced as those for the doses of 300 and 500 mg/kg valproic acid, which may indicate a dose-effect relationship as regards VPA and autistic symptoms .
4. Sage essential oil therapy in concentrations of 1 % and 3 %, has positive influences on autistic rats behavior, relieving anxiety and depression as well as improving short-term memory, effects especially visible 500mg/kg dose of VPA .
5. Prolonged subcutaneous administration of methionine in rats causes cognitive, social and affective-emotional deficits that may be considered relevant for schizophrenic symptoms . Such groups injected with methionine show deficiencies in learning ability and spatial memory, increased anxiety and depression, reduced scociability and disregard for social novelty .
6. Prenatal exposure to VPA results in a significant decrease of the specific activity of glutathione peroxidase in the temporal lobe, and significant increase of the concentration of malondialdehyde in the temporal lobe suggesting high oxidative stress .
7. Of particular relevance to the main theme of current research are the significant correlations registered between most behavioral indices and oxidative stress markers in autistic animal models generated by prenatal exposure to different doses of VPA .
8. Administration of methionine generates a significant decrease in the specific activity of GPx in the temporal lobe, and a significant increase in MDA levels compared to the

control group, indicating a sustained oxidative stress in the temporal lobe .

9. Statistically significant correlations were found between most behavioral indices and the oxidative stress markers dynamics in the temporal lobe.

10. Inhibition of the nucleus accumbens by administration of 6-OHDA results in a series of effects similar to schizophrenic symptoms, such as indifference to food or long-term memory deficiencies.

11 . Nucleus accumbens induced lesion by administration of 6-OHDA leads to increased oxidative stress in the frontal and temporal lobes .

12 . Nicotine administration in animals initially lesioned in the nucleus accumbens, generates an improvement in cognitive processes and decreases the level of the central oxidative stress, as demonstrated by the increase of the specific activity of superoxide dismutase and total antioxidant capacity, and the decrease of malondialdehyde concentration.

13 . Increased oxidative stress is automatically followed by severe cognitive deficits, the results obtained in the present study supporting the hypothesis that oxidative stress plays a crucial role in various central neural dysfunctions.