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Brain Oxidation Stress Caused by Isolation and Violation of Diurnal Cycle

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ABSTRACT. We have studied functional state of rat brain pro- and anti-oxidant system under stress caused by 40-day long isolation and violation of diurnal cycle. We have found that quantity of nitrogen oxide (NO) rises in rat brain cells, which is accompanied by an increase in the intensity of lipid peroxidation shown by a rise in the quantity of one of the primary and final products of this process – diene conjugates and thiobarbituric acid active compounds, including malonedialdehydes. At the same time, activity of anti-oxidant enzymes, such as superoxidedismutase and catalase is intensified at the initial stage of stress. Meanwhile, when stress prolongs the activity of these enzymes is drastically reduced, which may result in death of the cell. *© 2011 Bull. Georg. Natl. Acad. Sci.*

Key words*: brain, lipid peroxidation, stress, NO, superoxidedismutase, catalase.*

It is a known fact that any change in the environment causes stress reactions in an organism. Such changes include isolation and violation of the diurnal cycle for an individual. Isolation is an especially powerful stress factor resulting in a number of physiological and biochemical changes [1]. Similar changes were noted under violation of diurnal cycle [2].

Brief impact of stress factors actually strengthens cell metabolism and causes the organism to mobilize its resources. Meanwhile, the organism responds to prolonged stress with processes that cause changes in cell metabolism, such as increased intensity of free radical oxidation, changes in the quantity of Ca^{2+} in the cell, decrease in energy metabolism and protein synthesis, etc [3]. One of the characteristics of this change is activation of the lipid peroxidation process. Its intensity depends on the state of the anti-oxidant system in the cell [4]. Peroxidation is triggered by interaction of superoxide-anion radical created as a result of cell processes with other molecules [5]. Such molecules also include NO.

NO takes part in the processes that largely determine the vitality and activity of a cell and an organism as a whole. It is noteworthy that under certain circumstances NO is also involved in the formation of pathologic processes [6]. By reducing production and secretion of stress hormones NO protects the organism from the damage caused by stress [7]. By interacting with superoxide radical it is transformed into peroxide nitrite [8]. Peroxide nitrite stands out for its reactivity and conditions disintegration of cell structures, DNA, proteins and lipids [9]. Intensification of pro-oxidation processes that exceeds anti-oxidant capabilities of a cell further results in oxidation stress.

Anti-oxidant system of an animal organism is represented by a number of endogenic compounds and enzyme systems, especially superoxidedismutase (SOD) and catalase enzymes [10]. Their function consists in neutralization of superoxide-anionradical and hydrogen superoxide.

Our work aimed at studying changes in the activity

of lipid peroxidation process and anti-oxidant system taking place parallel to the change in the quantity of NO in laboratory rat brain cells under stress caused by isolation and violation of diurnal cycle.

Materials and Methods

The adult Wistar rats weighed in average 348±5g at the beginning of the experiment. The rats were 2 months old when the experiments were started. During the experiments the rats were given water and a standard laboratory chow *ad libitum*. In the animal facilities, air temperature was set at 20–21 °C, humidity was kept at 47%±2, and a 12h light 12h dark cycle was maintained. Rats were housed in cages (transparent polycarbonate, dimensions: 595×380×200mm, group housing and 210×380×190mm, single housing). The single-caged animals were placed in isolation 30–31 days prior to the beginning of the experimental period (socially isolated rats - SI-rats).

Behavior in the open field test is a widely used test of anxiety as well as of exploration and locomotor activity. The experiments were performed according to Barclay & Gibson [11]. Fractionation of brain tissue was performed as per Whittaker [12].

Quantity of NO was defined by the product of the reaction - ($NaNO_2$) between NO and molecular oxygen – O_2 [13]. We determined the concentration of thiobarbituric acid active compounds, including that of malone dialdehyde in the samples using thiobarbituric acid test at λ=532 nm wave length [14]. Diene conjugates of unsaturated fatty acids were determined using spectrophotometer [15].

Method for determination of catalase activity is based on the ability of hydrogen peroxide to create a complex with molybdenum salts. Intensity of its colorization is measured at λ=410 nm wave length [16]. The method used for defining activity of superoxide dismutase is based on the ability of the enzyme to compete with tetrazolium blue for superoxide anion radicals [17].

The data were processed statistically using t-test and ANOVA.

Results and Discussion

The locomotor activity parameters status of rats after protracted (30–31 days) social isolation was determined by open field test. Two groups of animals were examined and the differences between lines, i.e. the effects of social isolation compared with the group housed rats were analyzed. We found that SI-rats were significantly hypoactive (Fig.1), but showed no significant difference in center total distance ratios. These results indicate that isolated rats have similar levels of basal anxiety relative to normal rats.

Our previous experiments showed that isolation and violation of diurnal cycle caused inhibition of the enzymes participating in energy metabolism of rat brain mitochondria [18]. It was suggested that this process could have been initiated by quantitative changes in NO in the brain. Therefore, we studied the trend of the quantitative changes in NO in mitochondrial and cytosolic fractions of the brain under stress.

It has been found that isolation and violation of diurnal cycle among animals causes diverse changes in the quantity of NO. Particularly during brief stress (20 days) NO content practically remains the same. Its quantity increased only under prolonged stress – 30 and 40 days (Table 1). As we can see, on the 20th day of stress, when the quantity of NO practically remains unchanged, the intensity of peroxidation of lipids increases. Thus, the reason of such trend may consist in inhibition of the enzymes participating in energy metabolism of brain mitochondria, insufficient functionality of respiratory chain and generation of activated forms of oxygen [19].

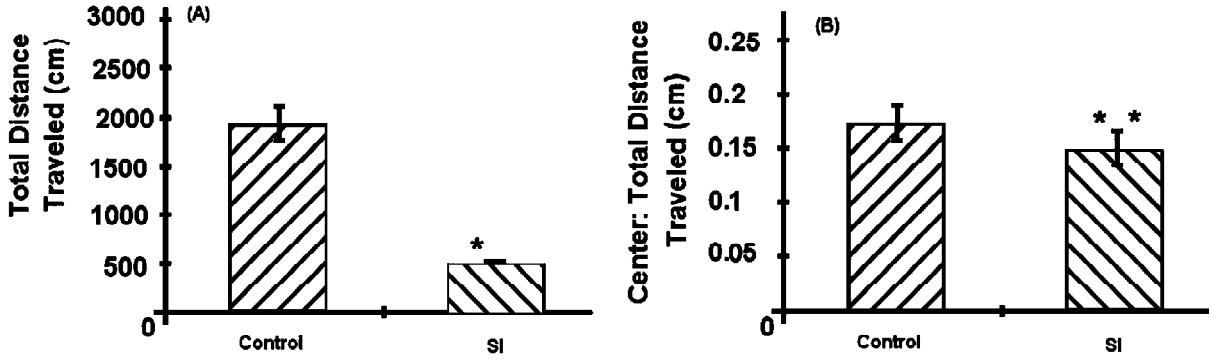

Fig. 1. Behavioral characterization of SI rats. Basal behavioral characteristics of rat were quantified. (A) Socially isolated (SI) animals were significantly hypoactive relative to control animals during a 15min session in the open-field task. (B) No significant difference in the ratio of distance traveled in the center/total was seen, indicating that isolated rate has similar levels of basal anxiety relative to control rats. Data represented are means±SEM of results from nine separate sessions. *$P < 0.05$, *t*-test compared with corresponding control groups. **$P > 0.05$, *t*-test compared with corresponding control groups.

Table 1

NO Quantity (μmol/ml) in Cytosolic and Mitochondrial Fractions of Hippocampus and Hemisphere Cortex

Different from the control: $*P \leq 0.05$, $**P \leq 0.001$

Duration of Stress	Mitochondrial Fraction		Cytosolic Fraction	
	Hippocampus	Cortex	Hippocampus	Cortex
Control	0.22 ±0.04	0.65 ±0.09	0.29 ±0.05	0.59 ±0.00
20 days	0.20 ± 0.01	0.68 ± 0.10	0.37 ±0.01*	0.60 ±0.02
30 days	0.48 ±0.07**	0.86 ±0.03*	0.48 ± 0.08*	0.78 ±0.06**
40 days	0.59 ±0.05**	1.04 ±0.09*	0.66 ±0.05**	0.93 ±0.09**

Taking into account that by inhibiting oxidizing phosphorylation NO reduces generation of ATP, which in its turn facilitates intensification of the lipid peroxidation process, we studied the trend of quantitative changes in thiobarbituric acid active compounds, including malone dyaldehyde (MDA) and diene conjugates of fatty acids under stress.

As we can see from Table 2, quantity of MDA – one of the final products of lipid peroxidation process – undergoes dynamic changes as a result of the stress caused by isolation and violation of diurnal cycle. In particular, 20 day stress caused virtually 100% growth in its quantity as compared to the control value, while 40 day stress resulted in a 5-fold increase in its quantity. Similar situation is observed in the case of cytosole. Interacting with protein molecules and nucleic acids, MDA facilitates the creation of inter-molecular bonds in them. This is how structural changes occur in various protein molecules, including enzymes [20]. It is suggested that this process is also reflected on the activity of enzymes of the antihoxidant system involved in cell metabolism.

Changes that occur in diene conjugates are different. Diene conjugates that are initial products of the lipid peroxidation process show about 2.5-fold increase under 20 day stress. Prolonged stress resulted in reduction of the value, and their quantity is reduced by about 30% as compared to the control value as a result of 40 day stress.

The results indicate that isolation of animals and violation of diurnal cycle among them resulted in a positive change in the intensity of lipid peroxidation, which can presumably lead to various anomalous cell processes.

Cell anti-oxidant system is known to be multicomponential. In particular, it includes superoxide dismutase (SOD) and catalase enzymes. SOD participates in transformation of O_2 into hydrogen peroxide, while catalase utilizes this product. This process is very important for normal functioning of a cell and its failure is one of the main reasons for death of cells. This is why we studied changes in SOD and catalase activity in rat brain cells under isolation and violation of diurnal cycle. The obtained data show that activity of mitochondrial SOD increases by 60% as a result of 20 day stress. If stress is prolonged to 30 days the activity of the enzyme decreases and after 40 days is 35% less than the control value. This

Table 2

Trend in the Change of Rat Brain Antioxidant Markers under Stress Caused by Isolation and Violation of Diurnal Cycle

Different from the control: $*P \leq 0.05$, $**P \leq 0.001$

Observation Object	Control	20 Day Stress	30Day Stress	40 Day Stress
Mitochondrial SOD (SOD act/mg protein)	15.79± 1.03	25.30±2.33**	11.99±1.45**	10.42 ±2.08**
Cytosolic SOD (SOD act/mg protein)	6.44± 1.14	9.10 ± 2.11*	7.90 ± 0.90*	2.72 ± 0.79**
Catalase (μmol H_2O_2 reduced/mg protein)	0.104± 0.05	0.143 ± 0.09	0.09 ± 0.01	0.054 ± 0.01*
Malone dyaldehyde in mitochondria (μmol/mg protein)	2.28±0.02	4.66±0.03**	6.71 ± 1.33**	10.24 ± 2.02**
Malone dyaldehyde in cytosole (μmol/mg protein)	1.15 ± 0.03	3.47± 1.02**	4.09± 0.88**	6.81± 1.64**
Diene conjugates (μmol/mg protein)	0.38 ± 0.02	0.88 ± 0.06**	0.56 ± 0.01*	0.12 ± 0.02*

indicates accumulation of excess peroxidation products, their damaging effect and degradation of anti-oxidant system. Activity of cytosolic isoform of SOD showed a similar trend. Changes were also noted in catalase activity. Isolation and violation of diurnal cycle among rats for 40 days reduces catalase activity by about 50% compared to the control value.

The obtained data indicate that isolation of animals and violation of diurnal cycle among them serve as factors for generation of stress and excess radicals. At the initial stage stress radicals may be neutralized by increasing the activity of anti-oxidant enzymes. However, prolonged stress may result in some irreversible processes that can result in complete dysfunction of the anti-oxidant stress followed by death of the cells and the entire organism.

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ბიოქიმია

იზოლირებითა და დღეღამური რიტმის დარღვევით გამოწვეული თავის ტვინის ოქსიდაციური სტრესი

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(წარმოდგენილია აკადემიის წევრის თ. ზაალიშვილის მიერ)

შესწავლილია ვირთაგვას თავის ტვინში პრო- და ანტიოქსიდანტური სისტემის ფუნქციური მდგომარეობა ცხოველების 40-დღიანი იზოლირებითა და დღეღამური რიტმის დარღვევით გამოწვეული სტრესის ფონზე. აღმოჩნდა, რომ ვირთაგვის თავის ტვინის უჯრედებში აღინიშნება აზოტის ჟანგის (NO) რაოდენობრივი მატება, რასაც თან სდევს ლიპიდების ზეჟანგური ჟანგვის ინტენსივობის გაძლიერებაც, რისი მაჩვენებელიცაა ამ პროცესის ერთ-ერთი საწყისი და საბოლოო პროდუქტების – დიენური კონიუგატების და თიობარბიტულით მჟავას აქტიური პროდუქტების, მათ შორის მალონის დიალდეჰიდის რაოდენობის ზრდა. პარალელურად, სტრესის საწყის ეტაპზე ანტიოქსიდანტური სისტემის ფერმენტების: სუპეროქსიდდისმუტაზასა და კატალაზას აქტივობა გაძლიერებულია, ხოლო სტრესის გახანგრძლივების შედეგად აღინიშნება ამ ფერმენტების აქტივობის მკვეთრი შემცირება, რაც უჯრედის დაღუპვის მიზეზი შეიძლება გახდეს.

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