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INVESTIGATION OF CORRELATION STRESS HYPERHOMOCYSTEINEMIA, IN RATS

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Abstract. Today it is generally accepted that the so called "stress" plays an important role in the process of aging. The main mechanism through which stress is involved in the aging process seems to be represented by reactive species generation. On the other hand, hyperhomocysteinemia, a risk factor in cardiovascular diseases, disturbs the normal endothelium functions and generates thrombosis through a mechanism in which reactive species seem to be also involved.
In our work, we have studied the influence of stress and hyperhomocysteinemia on antioxidant intra- and extracellular systems, in order to establish if there is a cumulative effect of these two parameters, in rats. Experimental stress was induced by reversing the normal cycle day/night (18oo – 6oo light) at the animals of experience for a 15 days period. Experimental hyperhomocisteinemia was induced by oral administration of methionine 2g/kg body weight, single dose daily, for a 15 days period. The activities of intracellular superoxide dismutase (SOD), glutathione peroxidase (GPx) and plasma total antioxidant status (TAS) were measured using Randox kit for manual use. The plasmatic concentrations of homocysteine were measured using a Roche standardized kit Diazyme. Our data show an increase in SOD activity, simultaneously with the decrease of GPx activity. The total antioxidant status and homocysteine levels have no significant changed.
In conclusion, subacute stress activates the antioxidant defense systems but doesn't influence the total antioxidant capacity and homocysteine levels, in rats.
Keywords: hyperhomocysteinemia, stress, total antioxidant status

Introduction

Today it is generally accepted that the so called **"stress"** plays an important role in the process of aging. The precise mechanism through which stress imbalances the oxidative cell status seems to be the generation of oxidative species. At cellular level, oxidative stress is considered a general decrease in reducing capacity of cell to detoxify the reactive species [1].

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Cells defend themselves against reactive species using intra- and extracellular systems. Among intracellular systems the most efficent defenders are enzymatic systems such as superoxyde dismutase, catalase, glutathione peroxidase and peroxiredoxine. At intracellular level, smalls molecules as ascorbic acid (vitamin C), tocopherol (vitamin E) and gluthation play important roles as antioxidant factors.

In the extracellular space, the antioxidant molecules are operating in order to preserve the oxidative balance. Total antioxidant status refers to all circulating species in plasma, including vitamin E, vitamin C, beta-carotene, uric acid, bilirubin, albumin as well as metal-binding proteins (e.g. ferritin or ceruloplasmin).

The effects of oxidative stress depend on its intensity, where cells can overcome mild stress and come back to normal state, or under higher or prolonged stress they can trigger apoptosis or even cellular necrosis [2].

On the other hand, it is also known that hyperhomocysteinemia is a risk factor in cardiovascular diseases, as it disturbs the normal endothelium functions and generates thrombosis even at slightly increased concentrations [3-5]. The mechanism through which homocysteine triggers these effects it is not precisely known, but it seems that reactive species generation plays an important role in these events.

In our work we have studied the influence of stress and hyperhomocysteinemia on antioxidant intra and extracellular systems, in order to establish if there is a cumulative effect of these two parameters, in rats.

Experimental stress was induced by reversing the normal cycle day/night (18^{oo} – 6^{oo} light) at the animals of experience for a 15 days period. Experimental hyperhomocisteinemia was induced by oral administration of methionine 2g/kg body weight, single dose daily, for a 15 days period.

The activities of intracellular superoxide dismutase (SOD), glutathione peroxidase (GPx) and plasma total antioxidant status (TAS) were measured in order to establish if stress and hyperhomocisteienemia imbalance the total antioxidant status. The plasmatic concentrations of homocysteine were also measured in order to establish if there is a direct correlation between these two cardiovascular risk parameters: stress and hyperhomocyteinemia.

Our data show an increase in SOD activity, simultaneously with the decrease of GPx activity. The total antioxidant status and homocysteine levels have no significant changed.

In conclusion, subacute stress activatsd the antioxidant defense systems, but doesn't influence the total antioxidant capacity and homocysteine levels, in rats.

Material and Method

We worked on four series of 10 adult male Wistar rats, weighting 150-200g. The animals from the different series were differently feed, as follow:

- Series **I** received food deprived of folic acid and B_{12} vitamin, but containing apples 5g/100g animal, carrot 5g/100g animal, black bread 10g/100g animal and barley 10g/100g animal, for 15 days, meanwhile animals were expose to stress by reversing the normal cycle day/night (18^{oo} – 6^{oo} light)

- Series **II** received standard food containing 0,5 mg/kg body weight folic acid and 10 mg/kg body weight B_{12} vitamin for 15 days, meanwhile animals were expose to stress by reversing the normal cycle day/night (18^{oo} – 6^{oo} light)

- Series **III** received food deprived of folic acid and B_{12} vitamin, but containing apples 5g/100g animal, carrot 5g/100g animal, black bread 10g/100g animal and barley 10g/100g animal, for 15 days, meanwhile animals were administered methionine 2g/kg body weight single dose daily by oral route.

- Series **IV** received standard food containing 0,5 mg/kg body weight folic acid and 10 mg/kg body weight B_{12} vitamin for 15 days, meanwhile animals were administered methionine 2g/kg body weight single dose daily by oral route.

Samples of blood were taken in order to determine oxidative stress parameters and homocysteine levels, initially and after 15 days of stress and methionine exposure.

The activities of intracellular superoxide dismutase (SOD), glutathione peroxidase (GPx) and plasma total antioxidant status (TAS) were measured using Randox kit for manual use.

Total homocysteine was determined in rat plasma using a Roche standardized kit Diazyme.

Results and discussions

Homocysteine levels which were determined initially and after stress exposure and metionine administration are presented in table nr.1

The registered data show that in the series III and IV, which received methionine, homocysteine concentrations are statistically significantly increased after 15 days of daily administration, as compared with the initial moment; in fact homocysteine concentrations exceed the upper concentration of the linearity domain. Homocysteine concentration presents similar levels for both series, despite the fact that the series IV received standard food which contains the appropriate amount of folic acid and vitamin B_{12}. It was expected that the presence of the two vitamins would decrease homocysteine leveles [6], but the repeated administration seems to overcome methionine metabolisation despite vitamins supplementation.

Homocysteine plasma levels determined after 15 day of induced stress didn't significantly modify as compared with the initial moment. The series II

	Homocysteine concentration (μM/ml) Mean ±SD	
No. = 10 animals/series	Initial	Final
Lotul I (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to stress for 15 days)	18.21±0.95	16.72±0.63
Lotul II (animals receiving standard food and exposed to stress for 15 days)	16.80±0.55	19.39±1.02
Lotul III (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to metionină administration 2g/kgc.b.w. single dose daily for 15 days)	16.3±0.78	>50
Lotul IV (animals receiving standard food and exposed to metionine administration 2g/kgc.b.w. single dose daily, for 15 days)	14.28±0.91	>50

Table no. 1. Concentration of homocysteine in rat plasma determined initially and after stress exposure and metionine administration for a 15 days period.

presents a mild (not statistic significant) increase in homocysteine level, despite the fact that it received standard vitamins food. It is known that vitamin supplementation is one way of decreasing homocysteine levels in human, and we would have expected homocysteine levels to remain at the same levels as at the beginning of the experiment. We can assume that subacute stress doesn't influence homocysteine levels.

The total antioxidant status determined in rat plasma is presented in table no.2.

After 15 day of stress exposure there were no significant changes in TAS levels at series I, meanwhile in series II there was a small not significantly TAS decrease. We suppose that subacute stress does not affect the total antioxidant capacity.

In the series III, IV which received methionine, TAS levels significantly decreased. As we expected,

	TAS (mM/L) Mean ±SD	
N = 10 animals/series	Initial	Final
Lotul I (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to stress for 15 days)	0.921±0.021	0.911±0.019
Lotul II (animals receiving standard food and exposed to stress for 15 days)	0.933±0.031	0.805±0.025
Lotul III (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to metionină administration 2g/kgc.b.w. single dose daily for 15 days)	0.908±0.022	0.760±0.024
Lotul IV (animals receiving standard food and exposed to metionine administration 2g/kgc.b.w. single dose daily, for 15 days)	0.897±0.036	0.756±0.041

Table no 2. Total antioxidant status determined initially and after stress exposure and metionine administration for a 15 days period

	SOD (U/ml) Mean ±SD	
N = 10 animals/series	Initial	Final
Lotul I (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to stress for 15 days)	219.08±15.6	945.90±45.2
Lotul II (animals receiving standard food and exposed to stress for 15 days)	211.22±18.8	946.22±37.5
Lotul III (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to metionină administration 2g/kgc.b.w. single dose daily for 15 days)	202.88±16.7	944.7±40.1
Lotul IV (animals receiving standard food and exposed to metionine administration 2g/kgc.b.w. single dose daily, for 15 days)	195.30±17.2	945.7±42.1

Table no 3. Superoxide dismutase activity, determined in red blood cells initially and after stress exposure and metionine administration for a 15 days period.

hyperhomocysteinemia disturbs the antioxidant balance by generating reactive species and decreasing the total antioxidant capacity.

The superoxide dismutase and glutathione peroxidase activities, determined in red blood cells, are presented in table 3 and 4 respectivly.

The registered data show that in all four series SOD activity increases. SOD is an enzyme involved in reactive species detoxification and the increase obtained in its activity indicates a prolonged exposure to these species, which in our case seems to be generated by stress exposure and hyperhomocysteinemia.

Obtained data show that in all four series GPx activity decreases. The decrease of GPx activity can be explained by the decrease of glutathione concentrations, the main cofactor of this enzyme. The diminished glutathione concentration is determined

	GPx (U/L) Mean ±SD	
N = 10 animals/series	Initial	Final
Lotul I (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to stress for 15 days)	67907±4356	40927±3598
Lotul II (animals receiving standard food and exposed to stress for 15 days)	68669±5132	49434±4003
Lotul III (animals receiving food deprived of folic acid and B_{12} vitamin and exposed to metionină administration 2g/kgc.b.w. single dose daily for 15 days)	60211±4926	34259±3897
Lotul IV (animals receiving standard food and exposed to metionine administration 2g/kgc.b.w. single dose daily, for 15 days)	64254±5067	39835±3524

Table no 4. Glutathione peroxidase activity, determined in red blood cells initially and after stress exposure and metionine administration for a 15 days period.

by its consuming as a consequence of the increased amount of reactive species. Stress exposure and hyperhomocysteinemia both generate reactive species, which disturb the antioxidant balance.

Conclusions

The obtained data show that methionine administration induces hyperhomocysteinemia, but subacute stress doesn't influence homocysteine levels.

The intracellular antioxidant capacity and plasma antioxidant capacity demonstrate a significantly decrease in values as hyperhomocysteinemia is installed.

Stress of mild intensity or subacute stress activates the antioxidant systems but does not modify the plasma total antioxidant capacity.

Subacute stress does not increase homocysteine levels. As a consequence, there is no correlation between stress and hyperhomocystenemia.

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