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Biochemical Features in Rats with Different Behavioral Activity under Conditions of Emotional Stress

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Adaptive capacities of the organism under conditions of emotional stress are determined by individual complex of anti-stress protective mechanisms. We studied the content of coenzyme Q10 and the spectrum of polyunsaturated fatty acids omega-3 and omega-6 in the serum, liver, and brain, and LPO intensity in the serum and liver of behaviorally active and passive rats under conditions of acute emotional stress. The differences in the formation of adaptive responses in animals with various behavioral activities were demonstrated.

Key Words: *emotional stress; coenzyme Q10; polyunsaturated fatty acids*

A wide range of physiological disturbances caused by negative emotions indicates that stress is a complex systemic response of the body. Experimental studies revealed individual differences in the resistance to the negative effects of stress in the mammals [5,9]. Behavioral activity in the open-field test is a reliable predictor of sensitivity to emotional stress in rats. In particular, active animals are prognostically more resistant to stress than passive animals [3].

The mammals develop many pathological consequences of emotional stress after stress load [1]. The most pronounced changes in behavior and heat production in rats accompanied by disorganization of circadian rhythms of these indicators were found within two post-stress days [4]. Thus, the study of certain basic metabolic changes, free radical oxidation and the markers of energy-dependent processes occurring in mammals after the negative emotional impact, is important for the development of new approaches to the prevention of stress-induced pathology. The study on the specificity of the development of biochemical post-stress disorders in individuals with different behavioral parameters characterized by differences in the systemic organization of physiological functions under normal and pathological conditions seems to be relevant.

Here we studied the dynamics of coenzyme Q10 content and spectrum of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) in the blood serum, liver, and brain as well as LPO intensity in the serum and liver in behaviorally passive and active rats at different terms after acute emotional stress.

MATERIALS AND METHODS

Experiments were performed on Wistar male rats (n=64) weighing 253.8±3.1 g. The experiments were carried out in accordance with the Regulations of the work using experimental animals, approved by the Ethics Commission of Anokhin Research Institute of Normal Physiology (Protocol No. 1, September 3, 2005), requirements of the World Society for the Protection of Animals (WSPA), and European Convention for the Protection of Experimental Animals.

Acute emotional stress was modeled by 12-h immobilization of the animals in individual plastic cages during night-time (21.00 to 09.00). Individual/typological characteristics of the rats were determined by open-field testing over 3 minutes [3]. Depending on the initial behavioral parameters in the open field, the animals were divided into "active" (n=32) and "passive" (n=32) differing by the mean activity in-

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dex (0.47±0.02 and 4.47±0.47 in passive and active rats, respectively). Later, behaviorally active and passive rats were divided into 8 groups (8 animals in each group): non-stressed (intact) active and passive animals (groups 1 and 2), active and passive rats subjected to stress exposure and decapitated immediately after emotional stress (groups 3 and 4), 1 day after exposure (groups 5 and 6), and 3 days after stress exposure (groups 7 and 8). The blood, liver, and brain were obtained after decapitation.

The levels of Q10 in serum, brain, and liver tissues were assayed as described previously [6]. Fatty acid composition in serum lipids, liver, and brain tissues as well as the serum and liver levels of TBA-reactive products were determined as previously described [7].

The significance of differences between groups was evaluated by using Student's *t* test; the differences were considered significant at $p<0.05$.

RESULTS

The content of Q10 in the serum and liver of passive animals was significantly increased in comparison with active rats in the intact group by 56 and 42%, respectively. There were no significant differences between the brain levels of Q10 in these groups (Fig. 1). During acute emotional stress, serum level of Q10 in active animals increased by 66% in the comparison with passive ones ($p<0.05$), in the liver, by 39%, and in the brain, by 57% ($p<0.05$). By day 3 of the restorative period, the serum and liver levels of Q10 were elevated in comparison with the control values both in passive animals (by 60 and 35%, respectively) and in active ones (by 85 and 68%, respectively, $p<0.05$). A tendency to return to pre-stress brain levels of Q10 was observed in both experimental groups.

The serum levels of linoleic acid (LA) increased in active and passive rats under conditions of acute stress in comparison with intact animals, while the levels of long-chain fatty acids C20:4, C22:5, C22:6 decreased; this can be due to LPO intensification in rat blood (Table 1). Thus, significantly lower content of TBA-reactive products was observed in the serum of active rats in comparison with passive ones in the intact group (by 3%), in the stress group (by 41%), and on day 1 of recovery (by 5%). However, serum levels of TBA-active products significantly increased in active animals (by 22%) in comparison with the passive ones by day 3 (Fig. 2). At the same time, serum levels of TBA-reactive products demonstrated the following dynamics: significant increase in comparison with control values on day 1 of recovery and marked decrease on day 3 of recovery. This indicates LPO intensification during stress in active and passive animals in the experimental groups, which persisted until day 1 of the recovery period. However, a distinct downward trend in this parameter to the pre-stress values was observed up to day 3 after the stress exposure.

The study of PUFA composition in the liver showed no significant differences between active and passive rats, but clear-cut predominance of omega-6

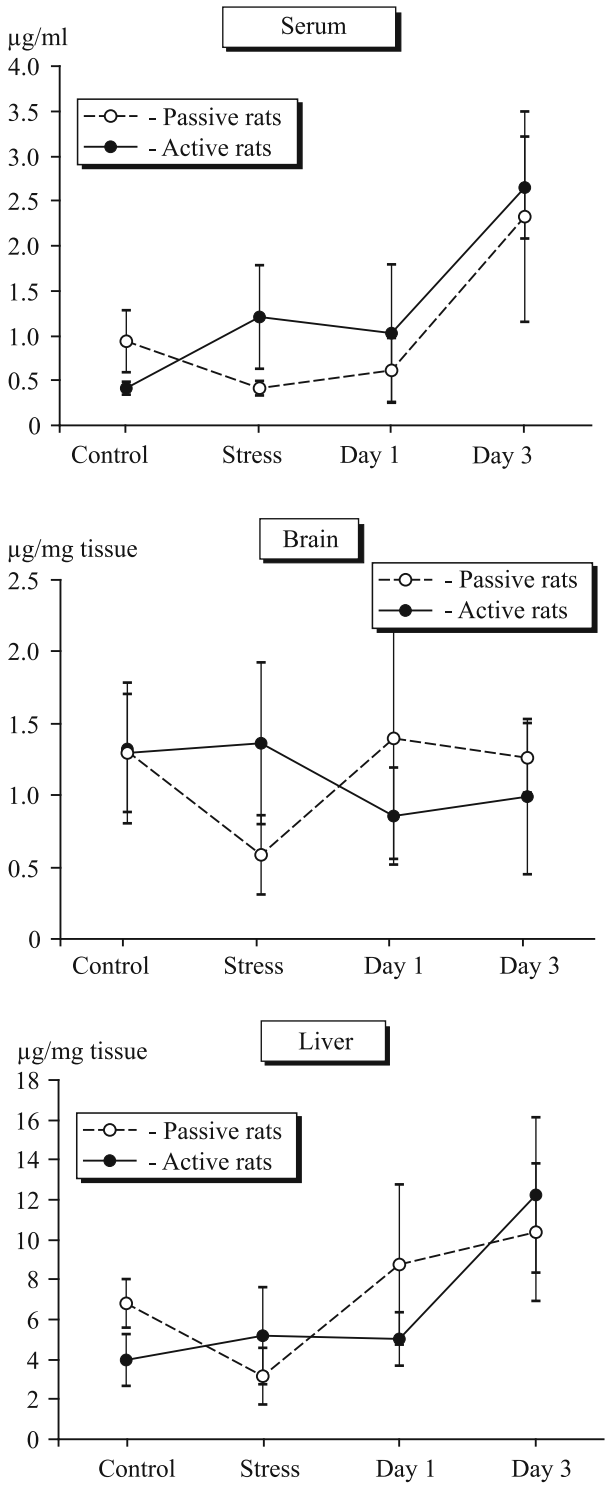

Fig. 1. Q10 levels in rat serum, liver and brain.

TABLE 1. Percentage of Omega-3 and Omega-6 PUFA in the Serum of Active and Passive Rats (*M*±*m*)

PUFA	Active rats				Passive rats			
	control	stress	day 1	day 3	control	stress	day 1	day 3
18:2 ω6	28.45±1.20	29.30±1.10	27.45±1.10	28.13±0.90	28.15±1.20	29.13±1.30	26.32±1.20	27.00±1.30
20:4 ω6	17.85±0.80	15.15±0.70*	16.45±0.60	16.89±0.70	17.03±0.60	15.43±0.90	15.23±0.70	17.50±0.80
22:5 ω3	0.87±0.05	0.66±0.09*	0.67±0.05*	0.83±0.06	0.64±0.04	0.35±0.03	0.46±0.03	0.61±0.04
22:6 ω3	1.43±0.06	0.93±0.08	1.05±0.08	1.12±0.10*	1.37±0.10	1.03±0.09*	0.99±0.08*	1.36±0.09
∑ PUFA ω3	2.76±0.15	2.25±0.10*	2.05±0.10	2.17±0.12	2.46±0.15	1.85±0.15*	1.83±0.10*	2.38±0.12
∑ PUFA ω6	46.73±2.20	44.99±2.10	44.36±2.10	45.41±2.00	45.58±2.00	45.03±2.10	43.88±2.00	44.91±2.20

Note. Here and in Tables 2, 3: *p<0.05 in comparison with controls.

TABLE 2. Percentage of Omega-3 and Omega-6 PUFA (%) in the Liver of Active and Passive Rats (*M*±*m*)

PUFA	Active rats				Passive rats			
	control	stress	day 1	day 3	control	stress	day 1	day 3
18:2 ω6	23.73±1.98	17.96±1.54*	18.26±1.90	20.35±1.95	23.42±2.18	16.98±1.45*	18.43±1.17	21.38±1.98
18:3 ω3	0.30±0.08	0.17±0.06	0.25±0.09	0.16±0.04	0.29±0.01	0.16±0.02*	0.15±0.02*	0.22±0.04
20:4 ω6	19.81±1.44	22.30±1.75	21.27±2.07	21.74±1.74	19.71±1.65	20.86±1.88	19.39±1.64	19.30±1.64
20:5 ω3	0.41±0.03	0.18±0.01	0.20±0.01	0.25±0.01	0.35±0.02	0.08±0.01	0.23±0.01	0.22±0.01
22:6 ω3	1.75±0.03	3.33±0.47	3.10±0.38	2.43±0.18	1.63±0.04	3.63±0.27	3.13±0.29	2.60±0.29
∑ PUFA ω3	2.93±0.10	4.16±0.38	3.95±0.40*	3.44±0.29	2.73±0.30	4.20±0.41	3.79±0.35*	3.47±0.40
∑ PUFA ω6	43.86±2.10	40.26±3.70	39.53±3.60	42.09±4.15	43.42±2.20	37.84±3.26	37.82±3.81	40.68±3.18

TABLE 3. Percentage of Omega-3 and Omega-6 PUFA (%) in the Brain of Active and Passive Rats (*M*±*m*)

PUFA	Active rats				Passive rats			
	control	stress	day 1	day 3	control	stress	day 1	day 3
18:2 ω6	1.10±0.08	0.96±0.07	1.26±0.10	1.05±0.05	1.15±0.09	0.58±0.04*	1.43±0.10*	0.78±0.05
20:4 ω6	10.86±1.00	9.69±0.82	10.24±0.92	11.74±1.19	10.93±0.80	10.86±0.8	9.39±0.60	12.34±0.90
22:4	2.37±0.06	3.78±0.10*	4.25±0.18	4.88±0.25	2.44±0.090	3.14±0.1	3.76±0.10	4.83±0.15
22:6 ω3	10.98±1.04	11.21±0.97	11.10±0.76	11.73±1.09	11.06±0.80	15.13±0.9	10.61±0.80	12.90±0.90
∑ PUFA ω3	11.82±1.05	12.64±1.01*	12.09±0.97	13.11±0.84*	11.87±0.90	16.60±1.1	12.17±0.90	14.37±0.90
∑ PUFA ω6	12.14±0.97	10.65±0.84*	11.50±1.24	12.79±1.30	12.08±0.90	11.44±0.8	10.82±0.80	13.12±0.90

PUFA is worthy of note (Table 2). At the same time, the study of the liver levels of LPO products in rats during emotional stress showed no statistically significant differences between active and passive animals. However, the level of TBA-active products was significantly increased in both groups during stress (by 30% in active rats and by 35% in passive ones) and decreased to pre-stress values up to day 3 of recovery after stress (Fig. 3).

Irrespective of stress exposure, the level of omega-3 PUFA in brain lipids remained the highest in comparison with other organs and tissues (Table 3). Comparative characteristics of PUFA composition revealed 50% reduction in LA in passive animals dur-

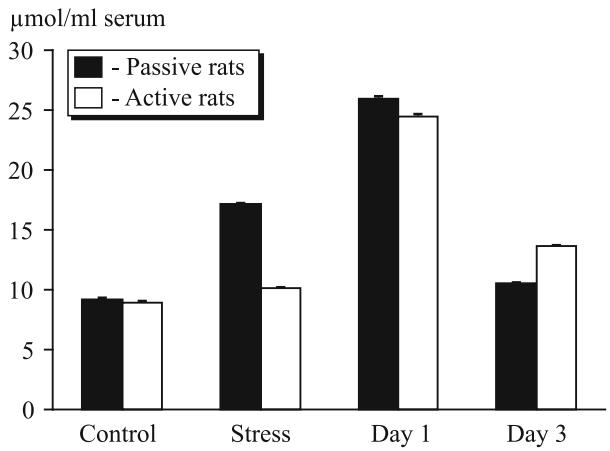

Fig. 2. Serum levels of LPO products in rats.

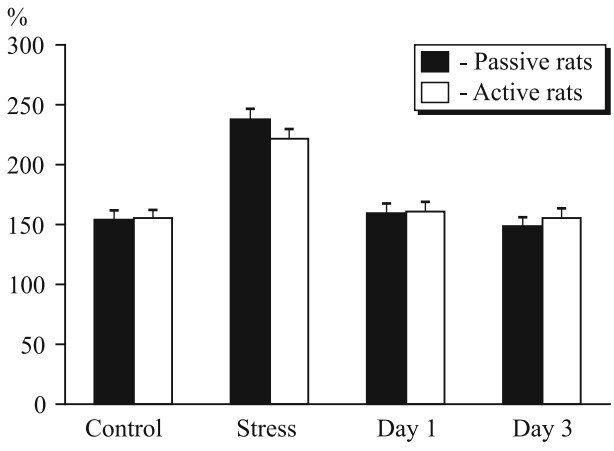

Fig. 3. Levels of LPO products in the rat liver.

ing the stress in comparison with the intacts. At the same time, the percentage of docosahexaenoic acid (DHA) providing the brain functions and subjected to rapid metabolism in tissues and organs, significantly increased. The total content of omega-3 PUFA was also higher during the stress in the brain of the passive animals in comparison with other experimental groups. Thus, emotional stress affects the activity of the synthesis of mega-3 PUFA in the brain, which may be associated with the action of adaptation mechanisms that contribute to maintaining DHA, the basic structural fatty acid of the brain. Changes in the composition of PUFA were negligible in active rats on post-stress day 1, and passive animals markedly increased LA levels and reduced DHA content. In this, total content of omega-3 PUFA and omega-6 PUFA did not differ significantly in both animal groups. The total content of omega-6 PUFA and omega-3 PUFA was increased in active rats on day 3 after emotional stress in comparison with stress period and post-stress day 1. Passive animals elevated omega-3 PUFA in comparison with post-stress day 1, but it remained lower than during stress. Total omega-6 PUFA were increased as in comparison with that during stress period and on 1 day after it.

These data suggest the formation of different adaptation mechanisms for overcoming the emotional stress in behaviorally active and passive animals. In particular, it is illustrated by increased Q10 production in the liver, which protective effect during activation of free radical oxidation was shown [10] and its increased serum level on post-stress day 3 with simultaneous reduction in LPO products. It should also be noted that stress impact is compensated faster in the brain, and adaptation to the emotional stress occurs already on day 1 of the recovery period. Since LA is a precursor of arachidonic acid and its derivatives, endocannabinoids, constitute the universal lipid signaling system controlling motor activity, emotional responses, and cognitive functions in all vertebrates [2,8], there are reasons to consider together the identified changes in Q10 levels and the intensity of lipid peroxidation. In this case, PUFA and the ratio of DHA to LA make a significant contribution as substrates, which act as major links of brain adaptation to stress.

REFERENCES

1. A. A. Bakhmet and E. V. Koplik, *Bull. Exp. Biol. Med.*, **153**, No. 5, 661-663 (2012).
2. A. V. Vasil'ev, N. E Sharanova, and S. N. Kulakova, *Vopr. Pitaniya*, No. 1, 4-11 (2014).
3. E. V. Koplik, *Vestn. Nov. Med. Tekhnol.*, **9**, No. 1, 16-18 (2002).
4. S. S. Pertsov, I. V. Alekseeva, E. V. Koplik, *et al.*, *Bull. Exp. Biol. Med.*, **157**, No. 1, 10-14 (2014).
5. K. V. Sudakov, *Selected Works*, **3**, *Emotions and emotional stress*, Moscow (2012), P. 534.
6. N. E. Sharanova, V. A. Baturina, A. V. Vasil'ev, and M. M. G. Gapparov, *Bull. Exp. Biol. Med.*, **151**, No. 6, 680-682 (2011).
7. N. E. Sharanova, S. N. Kulakova, V. A. Baturina, *et al.*, *Bull. Exp. Biol. Med.*, **154**, No. 3, 320-325 (2013).
8. Z. Cao, M. M. Mulvihill, P. Mukhopadhyay, *et al.*, *Gastroenterology*, **144**, No. 4, 808-817 (2013).
9. C. M. O'Mahony, G. Clarke, S. Gibney, *et al.*, *Pharmacol. Biochem. Behav.*, **97**, No. 4, 690-699 (2011).
10. R. S. Sohal and M. F. Forster, *Mitochondrion*, **7**, Suppl., S103-S111 (2007).