GENETICS

Multidimensional Assessment of Differences in Monoamine Metabolism in C57Bl/6 and BALB/c Mice

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Monoamine metabolism in the hypothalamus and striatum of BALB/c and C57Bl/6 mice (intact and stressed in the open field test) was studied using single- and multidimensional statistical methods. It is suggested that the revealed difference in neurotransmitter metabolism is associated with genetically controlled behavior of these animals under conditions of emotional stress. The results of discriminant analysis suggest that the regulation of monoamine metabolism during emotional stress is genetically determined.

Key Words: *inbred mice; brain; monoamines; stress*

Monoamines are known to play a significant role in emotional stress response [10]. Fundamental studies in recent decades revealed genetic heterogeneity of emotional stress response in mammals [3]. In view of this, the parameters of monoamine metabolism in animals with different stress response phenotype have to be reconsidered. Previous studies revealed interstrain differences in some metabolic parameters, however, these data are controversial [8], which can be explained by different models of stress, animals, terms of analysis, *etc.* Furthermore, taking into account the systemic character of neurotransmitter processes, single-dimensional statistical methods are of little promise for the analysis of their mechanisms. Therefore, our study aimed at the investigation of interstrain differences in monoamine metabolism in mice with different open field behavior, apart from traditional methods, employed the multifactorial linear discriminant analysis allowing some conclusion to be made about genetic differences in the function of this system.

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MATERIALS AND METHODS

The study was carried out on male C57Bl/6 and BALB/c mice (20-22 g) obtained from the Stolbovaya breeding center of the Russian Academy of Medical Sciences in the period from October to January. Two weeks before the experiments the animals (10 per cage) were maintained at a 12 h light /dark cycle (8.00-20.00 light period) with free access to water and food (standard laboratory chew).

Monoamine content in the hypothalamus and striatum of intact animals and after open field test [7] was determined as described elsewhere [5].

The data were processed statistically using Statistica and BMDP software. The significance of interstrain differences was assessed using the Kolmogorov—Smirnov test and the difference from the control was evaluated by Student's *t* test. The data were analyzed also by Fisher's discriminant analysis (Forward procedure with a sliding examination method) [1].

RESULTS

Monofactorial analysis of initial parameters in C57Bl/6 mice revealed a lower level of noradrenaline (NA) and

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higher levels of DOPAC and 5-hydroxyindolacetic acid (5-HIAA) in the hypothalamus (Table 1), and a lower level of homovanillic acid in the striatum in comparison with BALB/c mice. No significant interstrain differences were revealed in the intensity of monoamine metabolism (Table 2). Interstrain differences in DOPAC content, striatal DOPAC/dopamine (DA) ratio, and hypothalamic contents of NA and DOPAC became significant after testing in an open field (Table 1, 2).

The two mouse strains showed both similar (a decrease in NA content and DA turnover and an increase in hypothalamic DA content) and diverse (Tables 1 and 2) changes in monoamine metabolism after stress.

The results of monofactorial analysis allow to draw some conclusions about the relationships between neurochemical and behavior of parameters. Previous studies revealed an inverse correlation between the motor activity and brain NA content, which can be explained by higher rate of NA metabolism in active animals [12]. Our findings on the reduced NA content in the hypothalamus of C57Bl/6 mice agree with these data [12]. Brain serotonin content negatively correlated with locomotor activity and positively with emotional reactivity [4]. In our study, testing in an open field reduced the level of hypothalamic serotonin and 5-HIAA only in low-emotional C57Bl/6 animals. They also exhibited an increase in the striatal DOPAC/DA ratio which under conditions of unchanged content of these substances suggest more intense utilization of DA in active animals [10]. BALB/c mice characterized by similar DOPAC/DA ratios be-

TABLE 1. Effect of Open Field-Induced Emotional Stress on Monoamine Content (mg/kg) in the Hypothalamus and Striatum of BALB/c and C57Bl/6 mice ($M\pm m$, n=13-17)

Brain region	Monoamines and their metabolites	BALB/c		C57Bl/6	
		control	experiment	control	experiment
Striatum	NA	117.75±12.26	133.70±21.94	124.04±13.21	89.88±12.46***
	DA	1449.97±102.68	1444.83±133.42	1526.10±77.43	1458.27±129.17
	DOPAC	119.76±11.38	111.05±21.23	152.81±20.12	188.38±21.87++
	HVA	231.68±11.64	163.52±11.23**	180.77±16.42++	158.52±9.03
	5-HT	218.85±24.13	219.29±38.03	223.94±22.41	191.15±31.40
	5-HIAA	108.08±16.58	68.81±11.08*	113.08±16.00	98.95±15.97
Hypothalamus	NA	1552.68±84.18	1078.44±65.19*	1093.18±89.60++	734.35±64.74***+
	DA	125.50±17.27	227.62±18.17*	157.63±20.26	285.50±35.68**
	DOPAC	80.10±12.62	69.22±8.19	139.96±26.38++	143.87±16.91+
	HVA	107.95±17.47	116.02±14.30	182.04±34.08	95.23±10.89**
	5-HT	182.19±26.99	213.05±29.53	256.23±24.26	159.68±15.37**
	5-HIAA	337.86±66.90	342.37±44.61	440.80±38.91++	346.55±46.82

Note. Here and Table 2: *p<0.001; **p<0.01; ***p<0.01 in comparison with the control; +p<0.01; ++p<0.05 in comparison with BALB/c. 5-HT: serotonin.

TABLE 2. Effect of Open Field-Induced Emotional Stress on Neurotransmitter Metabolism in the Hypothalamus and Striatum of BALB/c and C57Bl/6 Mice ($M\pm m$, n=13-17)

Brain region	DA and 5-HT turnover	BALB/c		C57Bl/6	
		control	experiment	control	experiment
Striatum	DOPAC/DA	0.091±0.012	0.082±0.014	0.103±0.014	0.153±0.025***++
	HVA/DA	0.176±0.024	0.123±0.019***	0.122±0.013	0.125±0.015
	DOPAC+HVA/DA	0.266±0.029	0.205±0.023	0.226±0.024	0.278±0.037
	5-HIAA/5-HT	0.574±0.090	0.539±0.142	0.650±0.148	0.628±0.117
Hypothalamus	DOPAC/DA	0.866±0.160	0.334±0.063**	1.089±0.280	0.810±0.192
	HVA/DA	1.094±0.227	0.541±0.075***	1.432±0.282	0.549±0.170**
	DOPAC+HVA/DA	1.960±0.286	0.884±0.106**	2.521±0.506	1.359±0.309***
	5-HIAA/5-HT	2.635±0.555	2.226±0.534	2.218±0.472	2.754±0.484

TABLE 3. Results of Discriminant Analysis of the Content and Turnover of Transmitters in the Striatum and Hypothalamus of Intact and Stressed BALB/c and C57BL/6 Mice (n=13-17)

Brain structure	Discriminant function	Values		Prognostic efficiency, %		
		BALB/c	C57Bl/6	BALB/c	C57Bl/6	general
Hypothalamus (before stress)	NA	1552.7±84.8	1093.2±89.6			
	LC	0.013	0.008	73.3/73.3	76.5/76.5	75.0/75.0
	Constant	-10.483	-5.546			
Striatum (after stress)	DOPAC	111.1±21.2	188.4±21.9			
	LC	0.007	0.02			
	NA	133.7±21.9	89.9±12.5	78.6/78.6	82.4/82.4	80.6/80.6
	LK	0.028	0.002			
	5-HIAA	68.8±11.1	98.9±16.0			
	LK	0.004	0.020			
	Constant		-2.846	-3.606		
Hypothalamus (after stress)	NA	1078.4±84.8	734.4±64.7			
	LC		0.017	0.01		
	DOPAC	69.2±8.2	143.9±16.9	10.0/92.3	76.5/76.5	86.7/83.3
	LK	0.029	0.054			
	Constant	-10.888	-8.991			

Note. Significance level α=0.05. LC: linear coefficient: numerator - prognostic efficiency according to examination results in a learning sample, denominator — the same at sliding examination.

fore and after stress showed negative shifts in HVA concentration and HVA/DA ratio. The content of DOPAC and DOPAC/DA ratio in these mice were almost 2-fold lower than in C57Bl/6 mice. Opposite changes in striatal DA turnover in BALB/c and C57Bl/6 mice the notion of the role of the nigrostriatal system in the organization and regulation of motor behavior [13] and published data on direct relationships between striatal DA and locomotor activity [14].

According to the data of the discriminant analysis, the initial hypothalamic indices of both mouse strains could be discriminated with 75% probability (Table 3), which reflects the involvement of this structure in the regulation of autonomic and endocrine functions, emotionality, homeostasis, and other functions at rest [2]. The interstrain differences in the state of hypothalamic functions were also demonstrated in previous studies [11]. Striatal structures involved primarily in the organization and regulation of motor activity were similar in both strains (Table 3), which is in line with the data on similar motor activity in these strains at rest [6]. At the same time, poststress striatal levels of DOPAC, NA, and 5-HIAA and the hypothalamic contents of NA and DOPAC in C57Bl/6 and BALB/c mice were discriminated with the efficiency of 80,6 and 83,3%, respectively (Table 3). These findings suggest that emotional stress reactions are genetically determined and mediated by different mechanisms. This conclusion is confirmed by published data [9]. A sliding examination procedure applied to assess the stability of obtained discriminant functions only slightly reduced the efficiency of discrimination, which indicates high significance of discriminant indices (Table 3).

The conclusion about genetic determination of monoamine metabolism regulation during emotional stress has important psychopharmacological implications. Phenotypical peculiarities should be taken into account when using psychotropic drugs addressed to the monoaminergic systems. It also necessitates the development of a new approach to pharmacological correction of emotional stress response through regulation of its phenotypes.

REFERENCES

1. A. Afifi and C. Eisen, *Statistical analysis. Computer approach* [in Russian], Moscow (1982).
2. O. G. Baklavadzhan, *Physiology of Nervous System* [in Russian], Leningrad (1983), pp. 218-312.
3. D. K. Belyaev, *Vestn. Akad. Med. Nauk SSSR*, No. 7, 9-14 (1979).
4. A. V. Val'dman, M. M. Kozlovskaya, and O. V. Medvedev, *Pharmacological Regulation of Emotional Stress* [in Russian] Moscow (1979).
5. V. S. Kudrin, I. I. Miroshnichenko, and K. S. Raevskii, *Neuro-khimiya*, **7**, No. 1, 3-9 (1988).

6. S. B. Seredenin, *Pharmacogenetic Study of New Nootropic Compounds*, Abstract of Doct. Med. Sci. Dissertation, Moscow (1983).
7. S. B. Seredenin, B. A. Badyshev, and E. I. Kosenkov, *Byull. Eksp. Biol. Med.*, **105,** No. 3, 289-291 (1988).
8. S. B. Seredenin, Yu. A. Blednov, and B. A. Badyshov, *Science and Technique Reviews. Ecological Genetics of Humans* [in Russian], Moscow (1982).
9. T. P. Durkin, H. Hashem-Zaden, P. Mandel, *et al., Pharmacol. Biochem. Behav.*, **19,** 63-70 (1983).
10. Q. Pei, T. Zetterstrom, and M. Fillenz, *Neurosci.*, **35,** 133-138 (1990).
11. S. B. Seredenin, Yu. A. Blednov, B. A. Badistov, and N. A. Shevchenko, in: *Drug Dependence and Emotional Behavior: Neurophysiological and Neurochemical Approaches*, Ed. A. V. Valdman, New York-London (1986), pp. 49-77.
12. J. Slater, D. A. Blizard, and L. A. Pohorecky, *Pharmacol. Biochem. Behav.*, **6,** No. 5, 511-520 (1988)
13. S. Sudha and N. Pradhan, *Physiol. Behav.*, **57**, No. 6, 1061-1066 (1995)
14. A. Svensson, M. L. Carlsson, and A. Carisson, *J. Neural Transm. Gen. Sect.*, **101,** No. 1, 127-148 (1995).