The antistressor effect of diazepam has been explained on the grounds of its effect on the positive–negative reinforcement systems and the antinociceptive systems of the brain [4]. It has now been shown that the antinociceptive effect is not only a mechanism of central analgesia, but also an operant method of self-regulation and self-protection from the effects of stress, and it is realized both by a decrease in the intensity of the ascending nociceptive flow and by correction of the emotional-behavioral response [5]. Characteristically diazepam, through its action on antinociceptive systems, modulates exclusively the emotional-behavioral components of the response to stressor stimulation and depresses the activity of the negative reinforcement systems, while at the same time activating the zones of "positivity" [2].

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EFFECT OF CHRONIC STRESS ON BEHAVIOR, PHYSICAL STATE, AND BRAIN TYROSINE HYDROXYLASE ACTIVITY OF EMOTIONAL AND UNEMOTIONAL RATS

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KEY WORDS: chronic stress; emotionality; tyrosine hydroxylase; ulcers.

The role of genetic factors in responses of animals to stress has been established. High reactivity of "emotional" BALB/cI mice correlates with lower activity of the benzodiazepine receptors of the brain. In mice of "unemotional" lines (C57BL/10I, C57BL/6I) the affinity and density of the benzodiazepine receptors are significantly higher [11]. Differences have been found in the predisposition of rats of the August, Wistar, and Wag lines to emotional stress [7], but within each line animals predisposed and resistant to stress, with differences in their brain biogenic amine metabolism, can be distinguished [4]. Among populations of noninbred animals (cats, rats, mice) it is also possible to distinguish groups which differ considerably in their response to a stress situation and in their sensitivity to psychotropic drugs [2, 6].

On the basis of existing data showing the leading role of the brain catecholamine systems in the dynamics of the stress reaction [12], it was decided to estimate the activity of tyrosine hydroxylase (TH) in the brain, as a key component in catecholamine biosynthesis, together with behavioral and physical manifestations of exposure to chronic stress in rats with different levels of initial emotional and behavioral reactivity.

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TABLE 1. Indices of Behavioral Manifestations in Emotional and Unemotional Animals before and after Chronic Emotional Stress (M ± m)

Test situation	Behavioral manifestations	Control testing		Testing after stress	
		emotional rats	unemotional rats	emotional rats	unemotional rats
Open field	Latent period of crossing first square, sec	3,7±1,2	6,8±0,6	0,9±0,13†	4,7±0,7
	Vertical activity, number of standings	1,6±0,4	12,7±1,8	1,8±0,2	16,2±3,0
	Horizontal activity, number of times of crossing squares	86,0±10,3	64±5,4	174±12,7†	84,7±6,2*
	Number of defecations, number of boluses	4,3±0,6	2,0±0,3	5,7±1,6*	1,6±0,4
	No. of acts of grooming	4,8±1,7	1,8±0,7	5,6±2,0*	1,9±0,6
	No. of openings sniffed	1,9±0,3†	5,7±2,0	Absent	4,2±1,6
Dark chamber with openings	Latent period of first looking out	4,0±1,8†	7,9±2,1	Absent	6,3±0,4*
	No. of standings to look out	3,2±0,4†	12,1±0,8	"	13,5±2,1
Unfamiliar moving object	Level of anxiety (shown by avoidance reactions), conventional units	15,8±2,1	6,7±1,4	30,3±7,4†	7,9±2,7

*P < 0.05.

†P < 0.01.

Legend. Differences significant compared with control testing for each group.

EXPERIMENTAL METHOD

The emotional and behavioral reactivity of 30 noninbred male rats weighing 250-300 g was tested by the method described previously in experiments on "open field" type, in a dark chamber with openings, and by the response to an approaching object. The degree of expressiveness of the behavioral features was assessed quantitatively and on a point scale. The significance of differences was determined by the nonparametric U criterion [3]. A state of chronic emotional stress was simulated by prolonged (for 7 days) selective deprivation of fast sleep by "Jouvet's small areas" method [8]. Physical responses were assessed by counting the number of gastric ulcers and measuring the body weight and the weight of the adrenals and thymus. For the final investigation, four animals were selected from each group of "emotional" and "unemotional" rats, with the greatest differences in their behavioral manifestations. After exposure to stress for 7 days, emotional and behavioral reactivity was again assessed. The animals were decapitated 4 h later. Structures of the striatum and hypothalamus were isolated on ice. A 10% homogenate of the brain structures was obtained in 0.05M Tris-maleate buffer (pH 6.1), containing 0.2% Triton X-100. The homogenate was centrifuged at 12,000g for 15 min and the supernatant used as the source of the enzyme. TH activity was determined from the rate of formation of the reaction product (dopa) fluorometrically [13]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The main indices of behavioral reactivity of the emotional and unemotional rats are given in Table 1. The emotional animals differed by a significantly higher level of horizontal motor activity, accompanied by low values of investigative activity (vertical standing postures, venturing into the central zones of the "open field," investigation of the openings), by increased anxiety (as shown by the reaction of avoidance of frightening activities), and the intensity of visceral manifestations (defecation, urination). The TH activity of the emotional animals was significantly higher than that of the unemotional rats (Table 2), especially in structures of the striatum, in which it was twice as high ($P < 0.01$). These results are in agreement with data in the literature showing that genetically determined enzyme systems linked with biogenic amine metabolism are responsible for the type of an animal's emotional reactivity [10].

The behavioral and physical disturbances produced by stress for 7 days differed significantly in the animals of the two groups. No ulcers were observed in the unemotional rats although edema and hyperemia of the glandular part of the stomach were noted. The weight of the thymus was reduced in them by 39% but the weight of the adrenals was increased by 21%. The decrease in body weight amounted to 31% of the control. Many erosions were found in the glandular part of the stomach of the emotional animals and marked edema and hyperemia of the mucous membrane were present. In the nonglandular parts between two and four clearly distinguishable defects were present, consisting of ulcers measuring 1-2.5 mm^2 in area. The weight of the

TABLE 2. Level of TH Activity (nmoles/mg protein/15 min) in Structures of Hypothalamus and Striatum in Intact Animals and Animals Exposed to Chronic Emotional Stress, by Groups (M ± m)

Exptl. conditions	Emotional rats		Unemotional rats	
	striatum	hypothalamus	striatum	hypothalamus
Control	0,680±0,04	0,402±0,02	0,296±0,02	0,195±0,01
After stress	2,240±0,24*	1,881±0,28*	1,314±0,11*	0,816±0,03*

*P < 0.01.

Legend. Differences significant compared with intact animals for each group.

adrenals was doubled (increased by 100%) but the weight of the thymus was reduced by 70%. However, the reduction in body weight was less (26% of the control).

During behavioral testing the indices of the unemotional rats did not differ significantly from their original values (Table 1). The behavior of the emotional animals differed sharply from the original and showed a raised level of psychomotor excitation. A significant decrease in investigative activity and an increase in reactivity (the latent periods of active actions were sharply reduced) were observed and the avoidance reactions became distinctly affective in character. The horizontal activity was more than doubled, indicating a high level of anxiety. The rats had a tendency to leave the open space (they jumped on the wall of the "open field"). In the closed dark space they exhibited exactly the same tendency (the animals ran out of the chamber).

After chronic emotional stress TH activity was significantly increased in both groups of animals (Table 2). TH activity in the striatum and hypothalamus of the emotional rats was increased by 3.8 times and the original difference in the levels of enzyme activity in these parts of the brain disappeared (P > 0.05). TH activity in the unemotional animals was increased by 4.2 times compared with the control, but in absolute values it remained much lower than in the emotional rats. A significant difference remained between the levels of TH activity in the hypothalamus and striatum (P < 0.01).

The significant increase discovered in the level of TH activity after chronic emotional stress indicates intensified catecholamine metabolism in the hypothalamus and striatum, for the velocity of the TH reaction in the synaptosomal fraction reflects the general level of catecholamine biosynthesis in the brain in vivo, and after deprivation of fast sleep in rats (by Jouvet's method) a more rapid turnover of noradrenalin has been demonstrated (by injection of ^{3}H-noradrenalin into the cerebral ventricles and estimation of the rate of its metabolism) [9]. Recent investigations have shown [1] that the dopaminergic system of the striatum participates in the regulation of emotional behavior. Activation of the dopaminergic substrate of the striatum facilitates emotional reactions of negative character and aggressiveness. As a result of the higher TH activity in the striatum, the dopamine level in this structure was higher than in the hypothalamus which, besides dopaminergic nerve endings, contains noradrenergic endings and in which the noradrenalin level is higher than the dopamine level. Even prolonged (10 days) administration of dopaminomimetic agents was not accompanied by the development of tolerance to their behavioral effects in the rats; on the contrary, their emotional reactivity and aggressiveness were actually intensified [5]. The sharp rise in TH activity in the emotional rats in the striatum correlates with marked activation of emotional and motor reactivity, which may be connected with the inadequate compensatory activity of the restraining structures of the strio-pallidal system. The adrenergic system of the brain, especially of the hypothalamus, has the most direct relationship to regulation of stressor reactions of the animal and to activation of the hypophyseo-adrenocorticotrophic system. In turn, corticosteroids stimulate catecholamine synthesis and accelerate mediator turnover. During exposure to prolonged and severe stress, the mechanisms of adaptation may collapse as a result of exhaustion of the catecholamine or endocrine components, and the effects of such a collapse are manifested more clearly in emotional animals.

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ACTIVATION OF METABOLISM OF THE GABA SYSTEM IN THE CEREBRAL HEMISPHERES BY STRESS FACTORS

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Emotional and pain-induced stress (EPIS) is known to regularly cause activation of metabolism of the brain GABA system, which is expressed as a two-threefold increase in glutamate decarboxylase (GDC) and GABA transaminase (GABA-T) activity, and also as increased incorporation of labeled precursors into glutamate and GABA [3].

The study of the biological significance of this activation revealed that administration of γ-hydroxybutyric acid (GHBA), an end product of GABA, before EPIS largely prevents activation of the hypophyseo-adrenal system [6], the development of gastric ulcers [3-5], and disturbance of myocardial metabolism [4], which usually develop after severe EPIS.

On the basis of these observations it can be postulated that activation of brain GABA metabolism is a nonspecific mechanism which is realized during the action of various extremal stimuli on the body, and which by limiting the stress syndrome, prevents stress-induced injuries. To test this hypothesis, the basic indices of GABA metabolism were compared in this investigation in four groups (series) of animals.

The experiments of series I were conducted on Wistar rats weighing 180-200 g. EPIS was produced by the method of Desiderato et al. [4, 10], described previously, in the form of an anxiety neurosis, with a duration of 6 h. In the experiments of series II, conducted on similar rats, 2% formalin solution in a dose of 1 ml was injected subcutaneously into the region of the spine at intervals of 24 h for 2 days. Rats not subjected to any procedure served as the control to these two series. The experiments of series III were carried out on rabbits in which the descending branch of the left coronary artery was ligated by the usual method [1, 7]. The development of necrosis was confirmed by the ECG and the results of morphological investigations. The experiments of series IV were conducted on rabbits in which experimental aortic stenosis was created by the method described previously [2], resulting in a reduction of two-thirds in the area of cross section of the aortic orifice and leading to the development of compensatory hyperfunction of the heart. Animals on which thoracotomy was performed without ligation of the coronary artery and aorta served as the control for series III and IV.

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