BIOGENIC AMINES IN BRAIN STRUCTURES OF RATS OF DIFFERENT ZOOSOCIAL RANK DURING IMMOBILIZATION STRESS

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Resistance to emotional stress has been shown [8, 9] to be dependent on individual developmental factors. Keeping animals together in a living environment, and the characteristics of their goal-directed behavior for satisfaction of biological needs determine individual differences of each member and its hierarchic status. Many investigations have shown that the social and psychological characteristics of an individual can influence his physiological response to stress. Meanwhile the roles played by different structures of the CNS in the organization of the response to a stressor have not been finally elucidated.

The aim of this investigation was to study concentrations of biogenic amines and their metabolites in different brain structures: the mesencephalic reticular formation, the locus coeruleus, and the sensomotor cortex, in animals of different zoosocial rank and during immobilization stress.

EXPERIMENTAL METHOD

Experiments were carried out on 36 male Wistar rats weighing 200-250 g. Hierarchic relations between the animals were formed during competitive struggling for food [11]. The animals (three males in each group) were placed in transparent plastic boxes measuring 120 × 30 × 80 cm. Food was provided in a feeding bowl, approached by an inclined staircase 30 cm long. The hole through which food was obtained and the platform in front of it were such as not to allow three rats to obtain food at the same time. As a result of this, only one rat at each given moment could take food. Food (standard "kombikorm" granules) were put into the feeding bowl once a day. The food-getting skill of each animal was reinforced for 14 days. Next, under the same conditions and after 20 h of food deprivation, the test of competition for food was carried out – in this case the food was supplied daily for 1 h in separate portions (0.6-0.8 g) with an interval of 1 min. Because of restricted access to the food, a competitive struggle to obtain it arose between the animals. After each test lasting 1 h, the rats were fed to satiation, being allowed free access to food. During 14 days of testing the character of the food-getting behavior of the animals was discovered. The following stages of the food-getting process of each animal were recorded: mounting on the staircase, climbing to the platform, receiving a portion of food, the number of victories during the competitive struggle for food. Determination of the hierarchic rank of the animals was based on the success or otherwise of this food-getting behavior. Males having the maximal number of victories, essential to establish a dominant position in the group, were considered to be dominant. After statistical analysis of the parameters of food-getting behavior, the dominant and nondominant animals in each group could be distinguished. Next, 18 rats of both ranks (dominant and nondominant) were immobilized (IMO) in accordance with the scheme: 6 h IMO + 2 h resting + 16 h IMO + 8 h resting + 16 h IMO – by secure fixation of the limbs and head to a board. The remaining 18 rats of both ranks served as the control. After immobilization the rats were decapitated simultaneously with the control. The animals' brain was quickly removed

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TABLE 1. Content of Biogenic Amines and Their Metabolites in Parts of Brain of Rats Differing in Their Hierarchic Position (pg/mg wet weight of tissue, M ± m, n = 8-9)

Groups of animals	NA	A	DOPA	DA	DHPAA	5-HIAA	5-HT
	locus coeruleus						
Dominant	6621±664	447±96	663±106	226±15*	177±63	110±29**	102±20**,*
Nondominant	5978±1041	441±136	520±90	123±24	305±100	174±50	222±70
Dominant IMO	6376±1229	438±65	740±88	181±61	306±79	263±57	272±47
Nondominant IMO	5685±983	275±40	703±106	143±26	365±51	268±70	225±66
	Mesencephalic reticular formation						
Dominant	1733±265	183±33	162±29	72±20	63±12	25±5**,*	41±6**,*
Nondominant	1983±401	197±56	126±19	61±22	65±18	113±21	119±33
Dominant IMO	1818±268	107±16	191±29	37±6	126±50	272±35	232±27
Nondominant IMO	1369±270	138±19	124±30	45±15	66±14	230±53	148±36
	Sensomotor cortex						
Dominant	781±49	175±21	100±27	180±34*	29±5	52±19**	53±14**
Nondominant	697±70	152±30	81±17	71±28	53±28	76±17**	87±34
Dominant IMO	1041±224	192±54	158±36	135±48	82±61	196±48	185±51
Nondominant IMO	739±53	164±34	109±26	672±390	46±16	152±18	101±12

Legend. *) Significant difference between group of dominants and group of nondominants; **) significant differences between group of dominants and group of nondominants after IMO.

and frozen with dry ice. The mesencephalic reticular formation (RF; the oral part) and the region of the sensomotor cortex were removed by punching [16] from frozen sections 300 μ thick. The concentrations of noradrenalin (NA), adrenalin (A), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DHPAA), 5-hydroxyindoleacetic acid (5-HIAA), and serotonin (5-HT) were determined in these brain structures by HPLC with electrochemical detection [14], on a chromatograph of the "BAS" system (USA). Biogenic amines were extracted from the tissue with 0.1 M $HClO_4$ with the addition of 4 mM $NaHSO_3$. The mobile phase consisted of 0.15 M monochloroacetic acid (pH 2.4; from "Serva," Germany). The flow rate of the mobile phase through the column ("Biophase ODS"; 5 μ, internal diameter 4.1 mm, length 250 mm) was 1 ml/min. Oxidation of the monoamines took place on the surface of the working electrode at a voltage of 0.65 V. A glass-carbon electrode was used as the working electrode, Ag−AgCl as the comparison electrode. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The experiments showed that the dopamine content in the locus coeruleus and sensomotor cortex of the dominant rats was higher than in the same regions of the nondominant animals (Table 1). A higher serotonin content was observed in the mesencephalic reticular formation and in the locus coeruleus of the nondominant rats compared with dominant. After immobilization for 38 h, the dominant rats were found to have an increased serotonin content in all structures studied − locus coeruleus, mesencephalic reticular formation, and sensomotor cortex. An increased concentration of 5-HIAA was observed in the sensomotor cortex of the nondominant animals.

The results thus demonstrate unequal concentrations of catecholamines and serotonin in the brain structures of rats of different zoosocial rank studied. We know [2, 4] that establishment of hierarchic relationships is based on aggressive confrontations between animals. It has been shown [5] that animals genetically predisposed to dominance are characterized by high aggressiveness and low intensity of emotions. It has been claimed [16] that serotoninergic neurons perform an inhibitory role in various behavioral processes, including during aggressive behavior. Destruction of the nuclei raphe [13], which contain serotoninergic neurons, causes an increase in aggressiveness of laboratory animals. Conversely 5-hydroxytryptophan, a precursor of 5-HT, reduces aggressiveness in various experimental states. Meanwhile hierarchic relations are formed with the participation of serotonin [12]. In nondominant animals, for instance, the 5-HT concentration in the brain is higher than in dominant animals; destruction of the nuclei raphe, however, containing serotoninergic neurons and having terminals in many parts of the brain, led to a change in the animals' rank. Under our experimental conditions, the nondominant rats also had a raised 5-HT concentration in the brain structures studied than dominant rats.

Dominance is connected not only with aggressiveness; for dominant behavior to be manifested, motor activity is an important parameter [5]. It has been shown in mice that dominant males are characterized by a higher level of locomotion than subordinate animals. Meanwhile correlation exists between motor activity and activity of the serotoninergic system: the higher the motor activity the higher the 5-HT concentration in the animals' brain. It has been suggested that serotoninergic neurons trigger a motor response directed toward obtaining food [10]. Under our experimental conditions, dominant rats were distinguished by a higher 5-HT concentration in the sensomotor cortex and locus coeruleus.

It can be tentatively suggested that the mechanisms of dominance in behavior are related to many mediator systems, involved in the mechanism of a particular behavioral phenomenon. Changes in one mediator system can lead to changes in another. Correlation is known to exist between the dopaminergic and serotoninergic systems. For example, it has been shown [1] that DA can act as a pseudomediator, displacing 5-HT, blocking postsynaptic receptors of serotoninergic structures, and ultimately accelerating the catabolism of 5-HT, and thus lowering its concentration and increasing that of 5-HIAA – its breakdown product. In other words, an increase in the DA concentration under certain conditions leads to a decrease in the 5-HT concentration. Besides, DA increases the sensitivity of adrenergic receptors to NA [15].

After stress for 38 h a significant increase in the concentrations of 5-HT and its breakdown products was observed in the mesencephalic reticular formation, sensomotor cortex, and locus coeruleus of dominant animals, whereas in nondominant rats significant changes (an increase in 5-HIAA – breakdown product of 5-HT) was observed only in the sensomotor cortex. In all probability, with respect to the parameters studied, the level of stress of the nondominants was lower than that of the dominants. The reason may perhaps be that the formation of hierarchic relations in experimental groups during the first days takes place against the background of social stress [2, 4, 6], for both dominant and subordinate animals, the difference being that this stress reaction in the subordinates is stretched out over a longer period of time. Meanwhile we know [7] that the more the action of the stress-inducer is stretched out in time, the better will the mechanisms of adaptation be triggered. Meanwhile there is evidence [3] to indicate that stable activation of the pituitary-adrenal system during long-term administration of ACTH has an inhibitory effect on aggressive behavior and on the animals' tendency toward dominance and, in turn, it is under the regulatory influence of the serotoninergic system [3].

It can accordingly be postulated on the basis of the available data that DA and 5-HT in the mesencephalic reticular formation, sensomotor cortex, and locus coeruleus play an important role in the formation of hierarchic relations during the competitive struggle for food. Under conditions of immobilization stress the greatest changes in biogenic amine metabolism took place in dominant animals.

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MOLECULAR MECHANISMS OF DEVELOPMENT OF CARDIOVASCULAR PATHOLOGY

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The study of the complex pattern of biochemical changes at different stages of development of cardiovascular pathology reflects considerable metabolic disorders in the body. Among the causes of their appearance, a leading place is occupied by imbalance of the neuroendocrine systems [5]. All lesions must be manifested to some degree or other at the membrane level. This is the basis for a number of tests, for example, a raised blood enzyme level, when many tissue enzymes are found in the blood as evidence of serious tissue damage with cell destruction. In the native cell these changes must be determined by dysfunction of membrane mechanisms. The study of the mechanisms of intracellular warning, based evidently on functional responses of the cell, which are evidently universal [2], and effected through regulatory action, constitutes an important problem. Considerable interest of investigators has been attracted to the study of the regulatory function of the blood cells, by the use of delicate biochemical and biophysical methods. Some workers [1] observed stable differences of the dielectric constant of suspensions of blood cells in different forms of cardiovascular pathology. Pathological changes in ischemic heart disease (IHD) in stage II of essential hypertension (EH II), and in borderline hypertension (BH) are manifested at the cellular level by a difference in values of the complex dielectric constant of an erythrocyte suspension at a wavelength of $\lambda = 7.6$ mm. The molecular mechanisms responsible for these differences must probably be associated with catecholamine function in the adenylate cyclase system (ACS) of the cell.

The aim of this investigation was to study responses of the cell to procedures targeted on components of the ACS.

EXPERIMENTAL METHOD

Experiments were carried out on erythrocytes from healthy donors and patients with IHD, EH II, and BH. The value measured was the dielectric constant within the region of dispersion of free water ($\lambda = 7.6$ mm). Depending on the change in this parameter, the presence or absence of response of cell systems to factors acting on the protein components of ACS of the erythrocytes was established. The dielectric constant was measured on a microwave dielectrometer, specially modified for these purposes [6]. The error of measurement was 3% relative to

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