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CHANGES IN BIOGENIC AMINE METABOLISM IN RATS DIFFERING IN RESISTANCE TO STRESS, EXPOSED TO PRENATAL ANOXIA

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Cases have been described of the onset of pathology in childhood due to disturbances of the intrauterine fetal blood supply [6]. Experimental studies have shown that even transient anoxia of pregnant animals is accompanied not only by disturbances of growth and development of the progeny, but also by significant changes in orienting and conditioned-reflex behavior, and also by increased predisposition to convulsions under the influence of threshold doses of analeptics [2, 7]. The writers previously obtained data on the connection between predisposition of animals to audiogenic convulsions and brain monoamine (MA) metabolism [1, 3] and also on disturbances of investigative behavior and learning of animals due to neonatal administration of 6-hydroxydopamine (6-OHDA) [4, 9].

The aim of the present investigation was to study the effect of prenatal anoxia on biogenic amine (BA) metabolism in rats in the later period of postnatal development.

EXPERIMENTAL METHOD

Experiments were carried out on 58 rats aged 40 days, exposed to transient prenatal anoxia. On the 15th-17th days of pregnancy the rats were exposed daily for 2 h in a pressure chamber to a reduced air pressure corresponding to an altitude of 8000 m (220 mm Hg). The animals were kept in the animal house on a 12-h lightness and 12-h darkness cycle (8 a.m.-8 p.m. daylight, 8 p.m.-8 a.m. darkness) in cages and received granulated food (PK-120-7) and water and libutum. On the 30th day all the newborn animals were tested for their resistance to acoustic stimulation (electric bell, 96 db, 1.5 m). According to the results of testing, the experimental and control animals were subdivided into resistant (R) and predisposed (P) to audiogenic convulsions. The rats thus constituted four groups: groups 1 and 2 were resistant (19) and predisposed (eight) control animals and groups 3 and 4, which were R (20) and P (11) respectively, were subjected to prenatal anoxia. After 10 days the behavior of all the animals was tested in an open field measuring 1 m^2, divided into 100 squares. Observations on behavior lasted 3 min in the morning, and included determination of the latent period (LP) of the animals'

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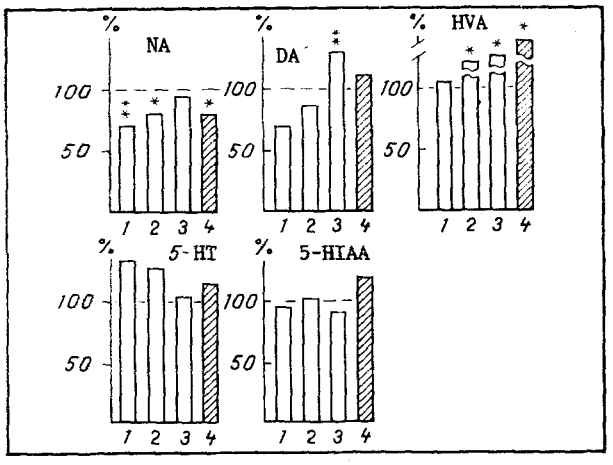

Fig. 1. Changes in concentrations of MA and their metabolites in brain structures and urine of rats exposed to prenatal anoxia (in % of their level in the control animals). Here and in Fig. 2: 1) neocortex, 2) hypothalamus, 3) brain stem, 4) urinary excretion. Here and in Figs. 2 and 3: *p<0.05, **p<0.01.

TABLE 1. Comparative Weight of Resistant and Predisposed Animals of Control and Experimental Groups on 40th Day after Birth

Group of animals	Number of animals	Average weight, g
1	5	123±10
2	8	93±11**
3	7	91±5*
4	6	81±12

Legend. *p < 0.01, **p < 0,003 compared with group 1.

TABLE 2. Concentrations of BA and Their Metabolites (in ng/g) in Brain Structures of Animals of Control Groups and Those Exposed to Prenatal Anoxia, Depending on Resistance to Acoustic Stimulation (data for anoxic animals shown as percentages of their own control)

Group of animals	Number of animals	Brain structure	NA	DA	5-HT	5-HIAA	HVA
1	5	Cortex	307±54	506±54	288±74	465±194	202±62
		Hypothalamus	509±62	352±59	232±121	504±175	217±78
		Brain stem	360±59	495±60	325±186	582±240	155±42
2	5	Cortex	255±26*	1507±286**	241±132	368±135	219±56
		Hypothalamus	422±49*	770±176*	372±180	582±154	124±20
		Brain stem	316±45	880±66*	390±167	737±388	218±150
3	6	Cortex	60	121	145	120	153**
		Hypothalamus	46*	53	131	123	200*
		Brain stem	59*	184	111	116	188**
4	6	Cortex	82	47**	132	105	57
		Hypothalamus	63*	92	92	83	172**
		Brain stem	89	110	89	103	66

Legend. *p<0.05, **p<0.01.

leaving the center of the field, the number of squares crossed, the number of rears, and the number of defecations. After the end of the experiments, some animals were subjected to biochemical tests. The rats were weighed and concentrations of MA and their metabolites in the urine and in three parts of the brain (neocortex, hypothalamus, and caudal part of the brain stem) were determined. Concentrations of noradrenalin (NA), dopamine (DA), and serotonin (5-hydroxytryptamine — 5-HT) were determined by a modified method [13], that of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) by the method in [8], and of the dopamine metabolite homovanillic acid (HVA) by the method in [10].

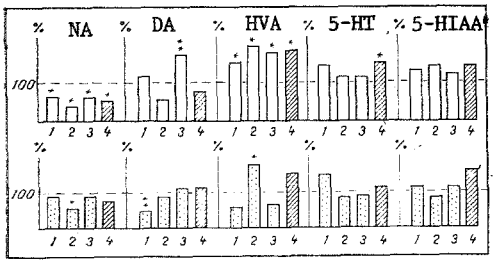

Fig. 2

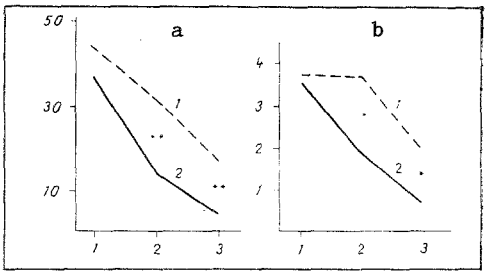

Fig. 3

Fig. 2. Changes in concentrations of MA and their metabolism in brain structures and in urine of resistant (top row) and predisposed (bottom row) rats exposed to prenatal anoxia (in % of their level in control animals).

Fig. 3. Dynamics of investigative behavior in an open field of rats exposed to prenatal anoxia and control animals over a period of 3 min. a) Number of squares crossed; b) number of rears. Abscissa, time (in min). 1) Control animals (n = 27); 2) anoxic animals (n = 13).

Concentrations of these substances in small volumes of urine were studied by the same methods, in our own modification. Fluorescence was measured on an FM-3MA fluorometer and "Perkin-Elmer" spectrofluorometer. The results were subjected to statistical analysis by Student's and Wilcoxon's tests.

EXPERIMENTAL RESULTS

Comparison of the weights of the animals exposed to prenatal anoxia and the controls on the 40th day after birth revealed considerable retardation of the anoxic rats in their body weight: the average weight of the control animals was 108 g and of the anoxic 86 g ($p<0.005$). A more detailed analysis of the groups of animals (Table 1) showed that anoxia had a greater effect on the weight of the resistant rats than the predisposed ($p<0.01$). However, an even greater difference in weight ($p<0.003$) was observed between the resistant and predisposed animals of the control group.

In Table 2 a significant difference in CA metabolism will be noted between the resistant and predisposed animals of the control groups, and a difference in the CA metabolism in the resistant animals of the control and experimental groups. In the first case this difference is expressed as a lowered NA level and a raised DA level in the predisposed animals of the control group by comparison with the resistant animals, in agreement with our previous data [1, 3] In the second case, lowering of the NA level also was observed in the anoxic resistant animals, most marked in the hypothalamus and brain stem, and accompanied by a considerable increase in the HVA concentration compared with resistant animals of the control group. In both cases the changes in CA metabolism are characterized by the fact that DA is metabolized mainly via the HVA pathway, and its transformation into NA is reduced. This is evidently connected with inhibition of activity of the enzyme DA-β-hydroxylase. These changes in metabolism also are reflected in the parameters of excretion of BA and their metabolites (Fig. 1). It will be clear from Fig. 1 that in animals exposed to anoxia the NA concentration was lowered and the HVA level in the urine raised, whereas the 5-HT and 5-HIAA levels in them did not differ from those in the control animals.

Detailed analysis of these data for the resistant and predisposed animals of the experimental and control groups separately showed that the changes described in CA metabolism after anoxia were more marked in the resistant animals (Fig. 2). Reduction of NA excretion in the urine of these animals, exposed to prenatal anoxia, was accompanied by increased excretion of 5-HT, although the 5-HIAA level was unchanged, indicating an increase in the intensity of 5-HT metabolism and reflecting the reciprocity of relations between the 5-HT and NA systems pointed out by the writers previously [5]. To understand the functional significance of these changes in BA metabolism arising under the influence of anoxia, experiments were carried out to analyze the behavior of the animals in an open field. It will be clear from Fig. 3 that in all animals exposed to prenatal anoxia the investigative activity was depressed, as shown by a change in the number of squares crossed and in the number of rears. This decrease in investigative activity was more marked in the predisposed animals than the resistant. Meanwhile, in predisposed anoxic animals LP of leaving the center of the field also was reduced, but the number of defecations was increased, evidence of their increased emotional reactivity.

The investigations showed a fall in the NA level in the cortex and hypothalamus, accompanied by elevation of the HVA level, in animals exposed to prenatal anoxia. This means that DA metabolism in the anoxic animals is switched from NA formation to another pathway, leading to an increase in HVA. This shift of DA metabolism evidently takes place not only in brain structures, but also in peripheral tissues, as shown by the urinary parameters. These shifts in the relationship between NA and DA synthesis were accompanied by disturbance of the animals' motor and investigative activity in an open field, similar to those observed by other workers in animals exposed to prenatal anoxia at the same period of embryogenesis [2], and also in animals with neonatal administration of 6-OHDA [4, 9], destroying catecholaminergic (CA) neurons. All these facts indicate that an important role in the disturbances of behavior of animals exposed to prenatal anoxia is played by changes in CA metabolism. In this connection it is important to emphasize that the animals were exposed to anoxia in the present experiments on the 15th-17th days of pregnancy, i.e., at that critical period when cellular differentiation of CA neurons takes place in embryos; at this stage of embryogenesis these neurons exert a trophic influence on the formation of target structures of CA innervation [11, 12, 14]. It remains uncertain why disturbances of CA metabolism under the influence of prenatal anoxia were more severe in the resistant animals, whereas disturbances of investigative behavior were more severe in the predisposed animals. It may be that in animals predisposed to the action of stressor agents the NA level lies on the boundary of the physiologically essential, and it therefore remains better preserved under anoxic conditions.

On the whole, the results confirm the view developed by the writers previously on the importance of an ontogenetic imbalance of activity of the NA and 5-HT systems of the brain in the genesis of disturbances of inborn forms of animal behavior.

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