GENETICS

Effect of Prenatal Stress on Proliferative Activity and Chromosome Aberrations in Embryo Brain in Rats with Different Excitability of the Nervous System

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Stress during pregnancy affects the morphogenesis of embryonal brain, its structural and functional characteristics, and behavior of the progeny. Genetic mechanisms of this process remain unclear. Cytogenetic characteristics of neuroblasts were analyzed in 17-18-day embryos of rats selected by threshold excitability of the nervous system in health and after emotional painful stress during the third trimester of pregnancy. The strains differed by the effect of stress on proliferative activity and chromosome aberrations in cells of the future hippocampus depending on the strain-specific characteristics of the nervous system excitability. This effect is regarded as an important component of epigenetic regulation of neurogenesis and behavior.

Key Words: *excitability of the nervous system; proliferation; chromosome aberrations; prenatal stress; rats*

Genetic and cytogenetic processes are regulated by the neurohormonal system [6]. Stress as a systemic nonspecific reaction to extreme environmental factors is associated with structural and functional alteration in the genome of somatic and generative cells [1]. The genomic effects of stress on the nervous system are investigated primarily at the molecular level. Matrix processes, expression of early genes and genes encoding hormones, receptors, stress proteins, and other bioactive substances are investigated [9,13-15].

The incidence of chromosome aberrations (CA) and proliferative activity in the brain have virtually never been investigated because of methodological difficulties and extremely low proliferative activity of nervous cells in adult animals. Embryonal brain is a convenient model for such studies. Prenatal stress affects the structure and functions of the nervous system, congenital and acquired behavioral reactions, reactivity to stress, which suggests the possibility of stress-induced modification of cytogenetic processes regulated by the neurohormonal system during neurogenesis [5,11,12,16]. The purpose of this study was to investigate the effect of emotional painful stimulation (EPS) of pregnant females selected by excitability of the nervous system on mitogenic activity of neuroblasts and the incidence of CA in the brain of their embryos.

MATERIALS AND METHODS

Experiments were performed on day 17 of embryo development. The rats with high (HT1 — 3.3 V) and low (LT1 — 0.8 and LT2 — 0.5 V) threshold sensitivities of the nervous system to electric current were selected from a population of Wistar rats by two selection programs [2] (41 generations for HT1 and 31 generations for LT1 and LT2). EPS was applied during the third trimester of pregnancy because this term corresponded to proliferation of hippocampal tissue in

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TABLE 1. Proliferative Activity of Brain Cells in Control Embryos and 24 h after EPS (*M±m*)

Parameter	HT1		LT1		LT2	
	control	experiment	control	experiment	control	experiment
Mitotic index, %	6.51±0.22	10.14±0.41*	4.86±0.42+	7.17±0.27*	4.74±0.32+	8.54±0.47*
Number of cells in:						
prophase	23.50±1.25	35.30±1.68*	11.87±3.87+	16.04±1.76*	16.04±1.56+	36.00±3.44*
metaphase	20.13±0.79	33.1±2.5*	16.3±1.8+	27.60±1.75*	16.4±1.2+	21.90±1.18*
anaphase	21.95±2.49	27.60±1.46*	17.14±1.67	25.50±1.67*	13.50±1.33+	25.30±3.87*
telophase	2.53±0.49	5.50±0.27*	1.07±0.31	2.80±0.47*	2.50±0.49	2.90±0.45

Note. *$p<0.05$ *vs.* respective control; +$p<0.001$ *vs.* HT1.

the embryo [8]. On day 16 of pregnancy, the females were exposed to electric current and light according to a stochastic scheme [10]: 6 nonreinforced and 6 reinforced (electric current: 2.5 mA, 4 sec) presentations of light stimuli in a special transparent cage with grid floor during 13 min. The control group consisted of unstressed females. The experimental and control rats were decapitated 24 h after exposure. The embryos (day 17 of gestation) were removed from the right and left uterine horns, fixed in Carnoy's fluid, and embedded in paraffin. Serial frontal sections (7 μ) of the endbrain including developing hippocampus were stained with hematoxylin according to Bemer, embedded in Canadian balm, and examined under a light microscope. The number of cells per mm^2 of developing hippocampus and the number of mitoses per 1000 nervous cells were determined; cells at various stages of cell cycle (prophase, metaphase, anaphase, and telophase) and CA (fragments and bridges) were counted and the mitotic index was estimated. Five embryos per group (at least 5 sections from each embryo) were examined. The results were statistically processed using Student's *t* test.

RESULTS

Cells of developing brain in low-excitable strain HT1 are normally characterized by high proliferative activity in comparison with those in rat embryos of highly excitable strains LT1 and LT2 (Table 1). Similar changes in two highly excitable strains close by the threshold excitability of the nervous system, but differing by the genetic background suggest that the observed differences are not accidental. Analysis of mitosis phases showed that the duration of early mitosis phases (prophase and metaphase) inversely correlates with excitability of the nervous system. The correlations with late phases (anaphase and telophase) were less pronounced. For instance, the number of cells in anaphase in HP1 embryos was virtually the same as in LT1 rats. The same was true for neuroblasts in telophase in HR1 and LT2 embryos. The mechanism of the observed relationship remains unclear. Mitosis is regulated by many genes responsible for cascade reactions controlled by hormones, transcription factors, cyclin-dependent kinases, *etc.* Characteristics of nerve cell membrane determining their excitability can also play a role. Differences in the structure of neurocyte membranes for rat strains used in our experiments were demonstrated previously [7]. The correlation between the duration of early phases and excitability becomes clear if we presume that the genes determining the synthesis of cyclin-dependent kinase controlling cell entry into mitosis are regulated by the nervous system. Both maternal and fetal nervous systems can participate in this processes, but the contribution

TABLE 2. Distribution of Cells in Developing Brain and Ratio of Early to Late Phases of Cell Proliferation Depending on the Type of Nervous System Excitability (*M±m*)

Parameter	HT1	LT1	LT2
Number of cells/mm^2 tissue	3.50±0.25*	2.50±0.09	2.76±0.07
Coefficient of cell cycle phases			
control	1.78±0.10	1.50±0.07	2.01±0.11
experiment	2.07±0.06+	1.55±0.09	2.06±0.08

Note. $p<0/005$: *vs.* LT2 and LT1; +*vs.* the control.

TABLE 3. CA in Embryos of Rats with Different Type of Nervous System Excitability ($M \pm m$)

Types of aberrations	HT1		LT1		LT2	
	control	experiment	control	experiment	control	experiment
Bridges	1.73±0.37	3.00±0.56	0.07±0.07	2.07±0.25	0.64±0.27	2.07±0.25
Fragments	1.73±0.37	3.00±0.56	0.01±0.01	1.20±0.28	0.08±0.07	0.93±0.23
Total	2.4±0.5	5.0±0.8	0.01±0.01	1.20±0.28	0.72±0.27	3.40±0.58

Note. All differences from respective controls are significant ($p<0.01$); all differences between control values in HT1, LT1, and LT2 strains are significant ($p<0.01$).

of each system can not be estimated. However, proliferation of neurons determined by strain-specific characteristics can result in different final number of cells. These differences were demonstrated in the early postnatal period [3] and in embryos (Table 2). The differences in the number of neurons in rats of different strains can determine behavioral peculiarities of examined strains. Stress alters the mitotic index irrespective of strain-specific characteristics of the nervous system (Table 1). Paradoxical stimulation of proliferation under the effect of EPS disagrees with published data on the inhibitory effect of stress on mitotic index in adult animals. This is probably characteristic of embryo development and is related to the ratio of pro- and antimitotic hormones in the developing organism. The effect of strain-specific characteristics of nervous system excitability manifests only in respect of the early to late phase ratio). This ratio increases in HT1 strain mainly due to changes in the duration of prophase and metaphase in dividing neuroblasts (Table 2). Nervous regulation of cyclin-dependent kinase genes can play the key role. CA were more often detected in HT1 rats (Table 3), which is in line with the data obtained on bone marrow of adult animals [4]. In highly excitable animals, stress increased the level of CA to a much greater extent. A similar effect correlating with pituitary-adrenal activation was observed in these strains in studies of the bone marrow during the postnatal period [4]. Different effects of stress on embryos can be explained by different activation of the pituitary-adrenal system, since corticosteroids easily penetrate through the placental barrier [5]. An important aspect in the ontogeny of the nervous system is the birth and death of neurons. The balance between these processes determines the definitive number of neurons. We showed that the effect of stress on cytogenetic characteristics of embryonic brain depends on strain-specific features. Presumably, EPS changes the density of cerebral neurons and modified brain function and behavior in the progeny. It is still more probable because, according to published reports, prenatal stress leads to a decrease in the number of neurons in rat spinal nuclei. Anterior commissure decreases in the progeny of females exposed to stress during the third gestation trimester [11]. Hence, the effects we observed can be regarded as an important component in the epigenetic regulation of neurogenesis and behavior.

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