Disorders in Early Embryonal Cellular Proliferative Properties Distort the Postnatal Formation of Nervous, Immune, and Endocrine Regulation Systems in a Newborn

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Disorders in the postnatal nervous, immune, and endocrine regulation systems were revealed in the progeny of rats irradiated during the preimplantation period of embryogenesis. These disorders persist till adult age. The direction of disorders confirms the hypothesis about memorization of changed proliferative properties of embryonal cells during the development of the (pro)endocrine system of a new organism. Memorization results in distortion of postnatal nervous immunoendocrine regulation: hypertrophy of the endocrine component and coadaptive underdevelopment of the nervous and immune components.

Key Words: *embryogenesis; early embryo; nervous regulation; immune regulation; endocrine regulation*

Total vulnerability of the nervous system of a future organism is formulated in behavioral teratology as follows: the aftereffects of exposure to any agent influencing the development of an embryo are manifested in deviations in the nervous system function of a newborn [9]. However, this assumption remains just a hypothesis until a universal biological mechanism is found, according to which any, including a nonspecific, disorder in embryogenesis can lead to defects in the nervous system of a newborn. The existence of such a mechanism (systemic) has been substantiated theoretically [7].

Ionizing radiation, nonspecifically impairing the proliferative properties of cells, has been chosen as a teratogenic factor [11]. The only acknowledged effect of ionizing radiation in early embryogenesis (before implantation of an embryo) is increased mortality of embryos but no disorders in the progeny born alive. Potential defects induced by radiation lead to death of the fetus or are completely repaired by the compensatory reactions [11]. These reactions are an immediate source of changes in the regulation systems of a newborn within the framework of the mechanism we hypothesize. Our task was to detect changes (and their trend) in the regulation systems of animals irradiated during the preimplantation period of embryogenesis.

MATERIALS AND METHODS

Pregnant Wistar female rats were exposed to γ-irradiation in a total dose of 2 Gy, 24 h a day during the first 6 days of pregnancy. Control group consisted of intact pregnant females. Newborn rats were kept with the mother for 4 weeks. On day 28, their sex was determined, and they were separated from the mothers. A total of 889 rat pups were examined: 487 exposed before implantation and 402 controls. Changes in the regulation systems of exposed ani-

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mals were detected by the differences in the parameters of their development and by the differences in the reactions of regulation systems of exposed animals to loading tests. Starting from day 28 of life, all parameters were analyzed separately for males and females.

Endocrine regulation of physical development was assessed by the time course of body weight gain and increase in size: from day 7 to day 56 the animals were daily weighed, their body length measured, and the ratio of body weight to square body length was calculated. On day 28, blood serum adrenocorticotropic hormone and insulin were radioimmunoassayed [4]. The endocrine regulation loading test was acute radiation sickness induced by acute exposure on day 100 of life. The body weight dynamics was recorded daily for 30 days after exposure.

The time course of the early development of the central nervous system (CNS) was assessed by the appearance of motor habits and signs of complete development of visual and acoustic analyzers during the first days of life [3]. For assessing the general status of the CNS, blood serum of rat pups was tested for autoantibodies to brain-specific protein S-100 and native DNA by enzyme immunoassay on day 42 of life [4]. The level of stress reactions was assessed by the relative weight of adrenals (organ weight/body weight) on days 42, 49, and 120 of life: in rats aged over 3 weeks the increment of adrenal weight depends on the stress-induced hypertrophy [5].

The immune status was assessed by the skin automicroflora test (SAMT) [3]. The immune reactivity test was carried out by intraperitoneal injections on days 28 and 35 of the following antigens: equine serum (0.1 ml/kg) or sheep erythrocytes (2×10^8 cells/animal). The measure of immune response was the time course of SAMT level after the antigen injection and the titer of antibodies to the antigen in the direct hemagglutination test on day 14 after injection. The readiness to allergic reaction after antigen reinjection was assessed by the peritoneal anaphylaxis test [8].

RESULTS

Body weight of prenatally irradiated rats was 10% less than that of control rats up to the age of 28 days ($p<0.05$), which can be regarded as an uncompensated result of decreased proliferative ability of embryonal cells. Starting from day 35 till the end of the observation period, body weight of irradiated rats was 10% higher than in the control ($p<0.05$): a stable compensatory stimulation of the total physical development of prenatally exposed rats was observed, which was excessive regarding the body weight increment. Body length of these rats was virtually the same as in the control, while the body weight/square body length ratio was 10-15% greater than in the control ($p<0.05$) throughout the entire observation period: excessive stimulation of the development of prenatally irradiated rats was abnormal and caused metabolic disorders. This was confirmed by a 2-fold increase ($p<0.01$) in blood level of hormones involved in the regulation of the body development and metabolism (adrenocortical hormone and insulin) in prenatally irradiated animals. Later this may cause some form of diabetes.

Starting from day 15 after acute irradiation on day 100 of life in doses 7 or 7.5 Gy, prenatally irradiated animals significantly ($p<0.01$) lagged behind the controls (irradiated in the same doses) in the postradiation body weight recovery; this indicates a more pronounced systemic reaction to acute irradiation. Adrenalectomized animals (with hypotrophic endocrine regulation) are characterized by a weaker systemic reaction to acute irradiation than intact animals [2]. The opposite differences in the reaction to irradiation stress in prenatally irradiated animals indicate hypertrophied endocrine regulation in comparison with the control. Does it lead to nervous and immune hypotrophy?

The dyschronism between the development of motor habits and manifestations of complete development of visual and acoustic analyzers was observed in prenatally irradiated rats in comparison with the control ($p<0.05$), which can be regarded as a disorder in the early development of the CNS and a prerequisite for its subsequent alteration [3]. The titers of autoantibodies to S-100 protein and native DNA were increased in 70% of prenatally irradiated rats but not in the controls. This indicates a decreased training ability of the nervous system of prenatally exposed rats [1]. The relative adrenal weight on day 42 of life was increased 1.8-fold ($p<0.01$), the initial differences from the control persisting till the end of the observation period (day 120). Thus, irradiation during the preimplantation period induced a decrease in the postnatal stress resistance [5]. Decreased training ability and stress resistance are the signs of nervous hypotrophy induced by irradiation before embryo implantation [7].

The level of SAMT on days 7, 35, and 56 of life was significantly ($p<0.05$) higher than in the control, with the peak on day 35, a week after weaning and discontinuation of immune agents obtained with maternal milk and a drastic increase in the consumption of antigens. A greater (3-fold) increase in SAMT level was observed in prenatally irradiated rats injected with antigens at the age of 28 days: the dependence of these animals on mater-

nal immune agents was greater, which is a sign of underdevelopment (hypotrophy) of immune reactivity. The signs of readiness to allergic reaction to reinjection of antigens were observed, and the antibody titers in response to antigens increased. Individual SAMT levels, antibody titers, and degree of peritoneal anaphylaxis strongly correlated (>0.85), i. e., the signs of immunoreactive hypotrophy induced by preimplantation exposure, belong to the same (dysontogenetically caused) total systems pathological process.

Our results confirm that impairment of the embryonal cell proliferative properties shifts the nervous-immune-endocrine regulation of a future organism, causing hypertrophy of the endocrine and hypotrophy of the nervous and immune components. This shift is a side effect of normal adaptive homeostatic response to any disorder in the embryonal cells proliferation; it is the cause of vulnerability of the nervous system in a future organism for any harmful prenatal exposure.

Congenital immune and endocrine disorders in the progeny are the common effects of any harmful prenatal exposure, which explains an increase in the incidence of all nervous, immune, and endocrine disorders observed virtually in all urban regions in the entire population, including infants. Exposure of pregnant women to numerous harmful environmental factors — deteriorating ecology, drugs, food additives, etc. — predisposes the newborns to various diseases, markedly potentiating the effects of postnatal exposure to environmental factors, for example, modern sugar-rich diets. Thus, the measures aimed at pregnancy protection can become the key to the improvement of population health.

Nervous hypotrophy (deteriorated training capacity of the nervous system and/or its decreased stress resistance) can result from abnormal proliferation of early embryonal cells at the period when tissue targets are not yet expressed in a developing organism. However, the proliferative properties of human cells vary due to their genetic peculiarities. Proliferative ability of cells of patients with severe hereditary diseases (Down's syndrome, ataxia-telangiectasia, etc.) is low [10], and the symptoms of these diseases include nervous and/or immune disorders. Therefore, genetically determined zygotic proliferative properties can be the primary mechanism triggering the development of the endogenic component of some mental diseases, at least those associated with a reduced training ability of the nervous system and its decreased resistance to stress.

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