Effect of Ultralow Doses of Antibodies to Erythropoietin on Antenatal and Postnatal Development of the Offspring

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We studied the effects of ultralow doses of antibodies to erythropoietin on antenatal and postnatal development of rat offspring. Daily administration of the preparation on days 1-6, 6-16, and 16-19 of pregnancy did not increase embryonic mortality and was not associated with congenital malformations, fetal growth retardation, high incidence of pathological changes in fetal organs, and delayed ossification (compared to control and intact animals). For evaluation of their embryotoxic effect manifested in the postnatal period ultralow doses of antibodies to erythropoietin were administered throughout pregnancy. The offspring of treated and intact rats did not differ in physical development, appearance of sensory and locomotor reflexes, locomotor, exploratory, and emotional behavior, and learning and adaptive capacities.

Key Words: *ultralow doses; antibodies to erythropoietin; prenatal and postnatal development; rat offspring*

Preparations of erythropoietin, the natural modulator of hemopoiesis, are used for the therapy of various diseases accompanied by anemia [7]. However, these preparations can produce the toxic effect [9]. The search for hemostimulators producing no side effects is an urgent problem. Homeopathic preparations containing active substances in ultralow doses hold much promise in this respect [9]. The original homeopathic preparation of antibodies to human erythropoietin stimulating the erythroid hemopoietic stem was synthesized at the Institute of Pharmacology and "Materia Medica Holding" Research-and-Production Company [6]. It is interesting to evaluate whether this preparation can be used for the therapy of pregnant women with anemia. Therefore, studies of the effects produced by this preparation on embryogenesis are of considerable importance. Here we studied the influence of this preparation on antenatal and postnatal development of rat offspring.

MATERIALS AND METHODS

Embryotoxic activity of the preparation manifested in the prenatal period was studied on 90 virgin female random-bred rats weighing 200 g. The animals were obtained from the Laboratory of Biological Models (Institute of Pharmacology) and kept according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). Before and during the experiments the rats were maintained in a vivarium under standard conditions and had free access to water and food (granular feed PK 120-3). Ultralow doses of potentiated antibodies to erythropoietin (PAB-E, C12+C30+C200) obtained using homeopathic potentiation technique were administered intragastrically to females on days 1-6, 6-16, and 16-19 of pregnancy (1 ml/rat). The day when spermatozoa in vaginal smears were detected was considered to be day 1 of pregnancy. Control rats received an equivalent volume of distilled water. The external appearance of rats, their behavior, and changes in body weight were recorded during pregnancy. Intact, treated, and control rats were killed by cervical dislocation on day 20 of pregnancy. Corpora lutea in the ovaries, implantation sites in the uterus, and alive fetuses were counted. The pre- and postimplantation mortality rates were determined [5]. The fetuses were removed and weighted. We measured the craniocaudal size, evaluated the sex distribution, and performed macroscopic examination. Some fetuses were fixed in Bowen's fluid to estimate the state of internal organs by the Dawson's method with modifications of A. P. Dyban. Other fetuses were fixed in alcohol and stained by the

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Dawson's method with modifications of A. P. Dyban to determine the state of the skeleton [4].

For evaluation of the effect of PAB-E on postnatal development of the offspring pregnant rats intragastrically received PAB-E in a daily dose of 1 ml. Control animals received an equivalent volume of the solvent. The offspring was obtained from 6 treated, 6 control, and 10 intact rats. We recorded the day of delivery and evaluated the number of rat pups in the litter, sex distribution, and index of survival [3]. Parameters of physical development of the offspring were monitored for 2 months. The appearance of sensory and locomotor reflexes during suckling period was studied in tests of turning on a flat surface, cliff avoidance, and muscular strength [5]. Behavioral characteristics of animals were estimated in the open-field test [1]. Learning ability and memory were assayed in the conditioned passive avoidance test [1]. Adaptive behavior was studied in the Handerson's stress avoidance test with modifications of A. P. Dyban.

The results were analyzed by Student's *t* test, Mann-Whitney *U* test, and Fischer's angular transformation [2]. The data obtained after autopsy of one female rat served as the independent variable. The average parameter was calculated for each litter.

RESULTS

Administration of ultralow doses of PAB-E during implantation (days 1-6), organogenesis (days 6-16), fetogenesis (days 16-19), and prenatal development produced no changes in the external appearance and behavior of animals. Changes in body weight of pregnant rats were similar in various groups. Only in animals receiving the preparation during organogenesisthe body weight tended to decrease ($p>0.05$). The rats of various groups did not differ in the number of corpora lutea in the ovaries, implantation sites in the uterus, and alive fetuses, average weight of fetuses, and craniocaudal size (Table 1). PAB-E did not affect the sex distribution in the offspring. Pre- and postimplantation mortality in the experimental group was similar to that in control and intact animals (Table 1).

Macroscopic examination of fetuses from various groups revealed no malformations. The ratio of fetuses with external hemorrhages was similar in all groups. Examination of internal organs showed that the animals did not differ in the incidence of hemorrhages in organs and tissues, dilation of the lateral cerebral ventricles, cholestasis, and hydronephrosis. The preparation administered in various periods had no effect on ossification of the skeleton.

When analyzing the effect of PAB-E on postnatal development of the offspring we found that pat pups in the experimental group were born in time. PAB-E had no effect on sex distribution and survival of the offspring (compared to control and intact animals). The body weight of pups born by treated, control, and intact rats was similar on days 5, 15, and 30 of life. However, this parameter tended to decrease in 2-month-old pups of females receiving PAB-E ($p>0.05$ compared to the control). Pups of various groups did not differ in other parameters of physical development (time of appearance of hair, eruption of teeth, opening of eyes and vagina, and downward displacement of the testes).

The percentage of 5-day-old animals displaying reflexes of cliff avoidance and turning on a flat surface was similar in all groups. In the test of muscular strength the female pups born by PAB-E-treated mothers de-

TABLE 1. Effect of PAB-E on Embryogenesis of Rats (*M*±*m*, *n*=15)

Parameter	Intact (*n*=10)	Period of pregnancy, days					
		1-6		6-16		16-19	
		control	PAB-E	control	PAB-E	control	PAB-E
Average number							
corpora lutea	11.8±0.4	13.5±0.7	13.6±0.6	13.6±0.6	13.7±0.5	13.6±0.6	13.1±0.5
implantation sites	10.9±0.4	12.4±0.6	12.2±0.4	11.8±0.8	12.6±0.7	11.5±1.1	10.5±1.1
alive fetuses	9.7±0.4	10.6±0.6	10.3±0.4	11.0±0.8	11.2±0.7	10.70±1.01	9.4±1.0
Death, %							
preimplantation	8.3±1.9	8.2±3.1	8.6±2.3	12.5±6.0	7.8±4.1	16.8±7.7	15.6±5.7
postimplantation	10.9±1.7	15.7±3.0	14.0±2.9	6.5±2.5	11.4±2.9	7.5±2.4	9.0±2.9
Mean weight of fetuses, g	2.4±0.1	2.40±0.03	2.3±0.1	2.30±0.04	2.3±0.1	2.30±0.03	2.4±0.1
Mean size of fetuses, mm	30.4±0.2	29.4±0.3	29.8±0.2	29.7±0.3	29.6±0.4	29.8±0.2	30.1±0.3

monstrated shorted time of hanging on a horizontal string than the offspring of control rats (7.08±0.02 and 10.56±0.07 sec, respectively, $p<0.05$). However, this parameter did not differ between the offspring of PAB-E-treated and intact animals (8.40±1.25 sec). On day 15 of life the pups of all groups demonstrated similar ability to hang on a horizontal string with hindlimbs and forelimbs and pull up and similar time of hanging.

The offspring of PAB-E-treated, control, and intact rats aging 1 month did not differ in horizontal and vertical locomotor activity, exploratory behavior, and emotional reactivity in the open-field test. The preparation had no effect on learning and memory in the conditioned passive avoidance paradigm in 2-month-old animals. The pups of various groups were capable of avoiding stress. The time spent in internal and external cylinders and period necessary to climb on a grid were similar in all rat pups. Emotional reactivity did not differ between the offspring of PAB-E-treated, control, and intact rats.

Our results show that treatment with PAB-E at various terms of pregnancy was not followed by an increase in the prenatal mortality rate, appearance of congenital malformations, fetal growth retardation, high incidence of pathological changes in fetal internal organs, and delayed ossification (compared to control and intact animals). The offspring of intact rats and females receiving the preparation during pregnancy did not differ in physical development, index of survival, appearance of sensory-and-locomotor reflexes, locomotor, exploratory, and emotional behavior, learning ability, and adaptive capacities.

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