EXPERIMENTAL BIOLOGY

Effect of Antibodies to Nerve Growth Factor and Serum Albumin on the Development and Behavior of Mice

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We studied physical development, behavioral characteristics, and learning capacity in the offspring of mice immunized with nerve growth factor and bovine serum albumin. High titer of antibodies to these factors in the blood of pregnant females determines high levels of these antibodies in the blood of their pups. These changes modulate physical development, behavior, and learning capacity of rat pups. The effects of these antibodies differed in the strength and directionality. Antibodies to nerve growth factor more markedly retarded physical development, reduced learning capacity, and considerably increased pain thresholds in animals.

Key Words: *nerve growth factor; serum albumin; antibodies; mouse behavior*

Recent studies showed that the concentration of antibodies to various antigens of the nervous tissue (*e.g.*, nerve growth factor, NGF) increases in the blood of mothers and newborns with nervous system dysfunction [2, 3,5]. However, the cause-effect relationship between an increased plasma antibody concentration and impaired development of the nervous system remains unknown. Here we studied a possible relationship between the increase in antibody concentration in pregnant females and their offspring. Besides this, we estimated the effect of antibodies on the development and behavior of newborn animals.

MATERIALS AND METHODS

Experiments were performed on 22 adult female BALB/c mice weighing 21±2 g and obtained from the Kryukovo-Tsentral'noe nursery. The animals were adapted to experimental conditions for 3 weeks and divided into groups. Group 1 (n=7) and 2 mice (n=7) were immunized with NGF and bovine serum albumin (BSA), respectively. Intact animals of group 3 (n=8) received injections of physiological saline and served as the control. The standard immunization schedule included 4-fold injections of 10 μg antigen in 100 μl physiological saline and complete Freund's adjuvant at 10-day intervals. NGF from bovine sperm [6] and commercial preparation of BSA (fraction V, Sigma) were used for immunization. Blood samples were taken after immunization. The level of antigens was measured. Then females were mated with males. The offspring of 3-4 females from each group was obtained after 4-6 weeks. BSA-immunized mice and control animals produced 12 and 15 pups, respectively. Feeding females and newborn mice were kept in individual cages. The animals were maintained at 21-23°C, 12:12-h light/dark cycle (daytime 7.00-19.00), and *ad libitum* water and food supply.

The pups were weighted 4 weeks after birth. Serum concentrations of antibodies to NGF and BSA were measured. Behavioral reactions of animals were assayed in the open field [4] and elevated plus-maze (EPM) tests. Pain sensitivity was studied in the hot-plate test.

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Learning capacity and level of anxiety were assayed in EPM elevated above the floor by 50 cm. The maze consisted of 2 open arms and 2 closed arms (45×10×40 cm) and central square (10×10 cm). The animals were placed in the central area of EPM. The head was directed toward the open arm. A piece of dry food was put at the far end of the open arm. The mice should find a piece of food and remember this place. Each pup was subjected to the EPM test 3 times a day. The animals were daily trained to find food until the appearance of significant intergroup differences (6 days). The animals were fed once a day in the evening, but had free access to water. The EPM behavior of pups was assayed for 10 min. Learning capacity was estimated by the time to find food. The level of anxiety was determined by the number of entries and time spent in open arms. Pain sensitivity was assayed in a hot-plate device. We recorded the time from placing the mouse on the plate (55°C) to licking the hind-limb.

The concentration of antibodies to NGF and BSA in the plasma was measured by enzyme immunoassay and expressed in optical density units [1].

The results were analyzed by Student's t test.

RESULTS

The titer of anti-NGF antibodies in group 1 pregnant females was 4-5-fold lower than in group 2 mice. It was probably related to variations in antigen immunogenicity for mice.

The concentration of plasma anti-NGF antibodies in 4-week-old pups of groups 1 and 2 was 0.40±0.17 and 1.66±0.59 optical density units, respectively. The level of antibodies to NGF and BSA in control pups did not exceed the baseline level (0.02 optical density units). No correlation was found between the blood concentration of antibodies in pregnant females and pups. By the end of the second month of life the concentration of antibodies to NGF and BSA in group 1 and 2 pups decreased and practically did not differ from the control. Therefore, the rise in antibody concentration in the blood of pregnant females was accompanied by a transitory increase in their level in pups. The content of antibodies in the blood of pups returned to normal by the end of the second month of life.

The mean weight of 1-month-old pups was 16.3± 2.2 (control), 10.7±4.0 (group 1), and 13.3±1.47 g (group 2).

The weight of young animals of groups 1 and 2 was much lower than in the control group ($p<0.01$). It should be emphasized that body weight was minimum in animals with high titer of antibodies to NGF. A negative correlation was found between the concentration of anti-NGF antibodies in the blood and body weight of young animals ($r=-0.91$, $p<0.05$). Young animals of group 1 with the highest level of anti-NGF antibodies (0.7 optical density units) were less viable and died by the third day of the study. These data indicate that anti-NGF antibodies circulating in the blood during embryogenesis and early postnatal ontogeny cause growth retardation in mice. Antibodies to BSA had a similar, but less pronounced effect.

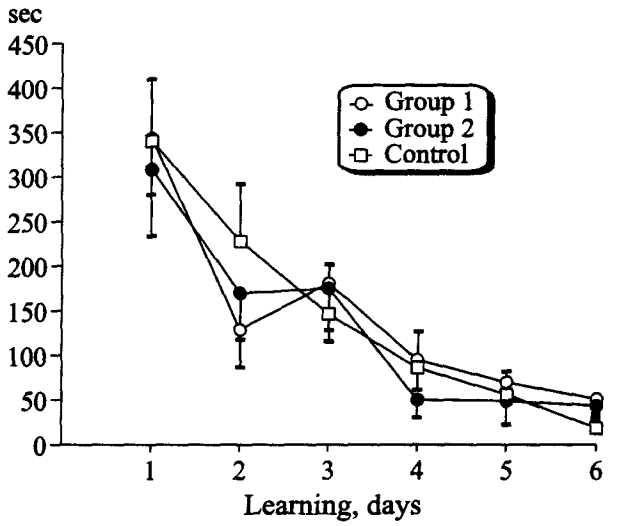

Fig. 1. Time to find food in the elevated plus-maze for mice of various groups.

On day 6 the mice of groups 1 and 2 need more time to find food in EPM compared to control animals (Fig. 1). It should be emphasized that this period was maximum for group 1 animals.

After 3 weeks repeated testing in EPM showed that group 1 animals need more time to find food than control mice ($p=0.007$). Our results suggest that the skill was partially preserved in control mice, but completely lost in animals with high level of anti-NGF antibodies. Group 2 mice were intermediate by this parameter. However, no significant differences were revealed between group 2 mice and control animals.

Anti-NGF antibodies circulating in the blood of experimental animals during embryogenesis and early postnatal ontogeny impair their learning capacity. Antibodies to BSA had similar, but less pronounced effect.

TABLE 1. Open Field Behavior and Parameters of Hot-Plate Test ($M±m$)

Index	Control	Group	
		1	2
Number of crossed squares	19.2±3.8	41.3±9.2*	36.8±7.7
Latency, sec	54.2±6.3	128.0±120.4*	63.6±11.5

Note. *$p<0.05$ compared to the control.

We recorded the number of entries and the time spent in open arms of EPM. These indexes reflect the level of anxiety [4]. There were no differences in EMP behavior between different mouse groups. These data suggest that antibodies have no effect on the emotional and stress responses of animals.

Locomotor activity in the open field in group 1 and 2 mice far surpassed that in controls (Table 1). Therefore, antibodies against NGF and BSA present in the blood during embryogenesis and early postnatal ontogeny increase locomotor activity of animals in the open field. It can be hypothesized that increased locomotor activity of mice is not related to emotional changes.

Pain thresholds in group 1 mice significantly surpassed those in group 2 animals ($p<0.006$, Table 1).

Our results confirm the hypothesis that the increased in blood concentration of anti-NGF antibody in pregnant women impairs brain development in children. Activation of the immune system (*e.g.*, immunization of females with BSA) probably has an adverse effect on CNS development in the offspring.

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