Immune System in Vasopressin-Deficient Rats during Ontogeny

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Morphofunctional immune disorders were revealed in vasopressin-deficient Brattleboro rats with diabetes insipidus during ontogeny. We observed a permanent decrease in the number of blood lymphocytes, increase in neutrophil count, reduced activity of macrophages, early involution of the thymus and spleen, and suppression of antibody production. These changes reflect impaired general resistance of these animals.

Key Words: *diabetes insipidus; blood count; thymus; spleen; humoral immune response*

Immunoregulatory functions of vasopressin are related to expression of membrane hormonal type V_1 receptors [7,9,15] and ectopic synthesis of this hormone in immunocompetent cells [8,10,13]. Vasopressin stimulates the synthesis of immunoglobulins and other biologically active proteins in lymphocytes [15], promotes proliferation of T lymphocytes, and modulates the development and differentiation of thymocytes [11]. Single administration of exogenous vasopressin affects the count of antibody-producing cells (APC) in the active phase of the immune response [3].

The deficiency in the synthesis and secretion of vasopressin should be accompanied by dysfunction of the immune system. In the present work physiological parameters characterizing the immune system in Brattleboro rats genetically incapable of synthesizing vasopressin were studied in various periods of life.

MATERIALS AND METHODS

Experiments were performed on Brattleboro and WAG rats carrying mutant and normally expressed gene for vasopressin, respectively. The age of animals was less than 1 year. Blood smears were fixed with methyl alcohol and routinely stained to estimate differential leukocyte count [6]. Functional activity of neutrophils was determined by oxygen radical generation and reduction of nitro blue tetrazolium (NBT). To evaluate properties of macrophages a 1-day culture of peritoneal cells was incubated with sheep erythrocytes. The numbers of phagocytizing macrophages were compared in intact rats and animals receiving intraperitoneal injection of tumor necrosis factor-α (TNF-α) [14]. The weight of immune organs was divided by body weight and multiplied by 10^3 to estimate weight indexes. The rats were intraperitoneally immunized with 0.5 ml 50% suspension of washed sheep erythrocytes. The primary immune response was determined by the method of local immune hemolysis. APC in the spleen were counted 4 days after immunization [1]. The animals were immunized 2 times at a 2-week interval to study the secondary immune response. Goat antiserum against rat IgG was added to the incubation medium 4 days after repeated immunization to detect APC.

The average intensity of water consumption was estimated daily for 5 days. In individual Brattleboro rats 24-h water consumption was 25-100% of body weight and did not surpass 5% of that in WAG rats.

The results were analyzed by Student's *t* test.

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RESULTS

Brattleboro rats have severe diabetes insipidus and are characterized by intensive consumption and excretion of water due to the absence of blood vasopressin [5]. Under normal conditions vasopressin maintains osmotic equilibrium by regulating water reabsorption in the kidneys. Therefore, changes in drinking behavior serve as an external criterion for alterations in the hormone status [14]. We studied the dynamics of mean daily water consumption from the start of self-feeding in rat pups to the appearance of irreversible age-related changes. The periods of life were selected taking into account the sequence of physiological processes in rats during ontogeny [2]. Mean daily water consumption was repeatedly measured in homozygote Brattleboro rats aging 1, 3, 6, and 12 months (Fig. 1). The intensity of water consumption did not differ in animals aging 3 and 6 months, but decreased at the age of 12 months. This symptom of diabetes insipidus is an individual characteristic that reflects stabilization of compensatory osmoregulatory mechanisms in the absence of vasopressin.

Other changes were observed in rats with a genetic deficiency in vasopressin synthesis during ontogeny. White blood cell count significantly differed in Brattleboro and WAG rats (Table 1). The number of white blood cells was presented in percents of the total leukocyte count. Functional activity of neutrophils and macrophages was determined by the percentage of active cells. Weight indexes were expressed in percents of body weight multiplied by 10. The number of APC was calculated per 10^6 nuclear splenocytes. In Brattleboro rats aging 6 and 8 months the number of neutrophils was much higher, while the count of lymphocytes was lower than in WAG rats. At the age of 6 months the monocyte index in Brattleboro rats surpassed that in WAG rats. The count of eosinophils in 8-month-old Brattleboro rats was 2-fold lower than in

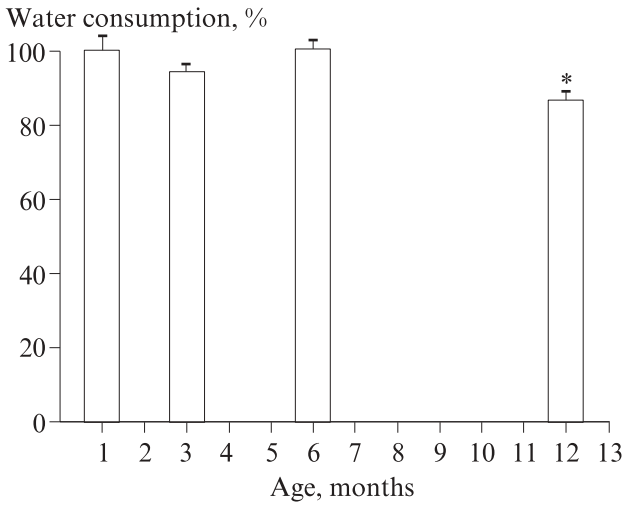

Fig. 1. Age-related changes in mean daily water consumption in homozygote mutant Brattleboro rats (n=45). Repeated measurements in various periods of life. The data are expressed in percents of water consumption at the age of 1 month. *p<0.001 compared to previous term.

TABLE 1. Morphological Indexes and Reactivity of the Immune System in Rats of Different Ages ($M\pm m$, n=4-12)

Indexes	Age and strain of animals					
	1 month		6 months		8 months	
	WAG	Brattleboro	WAG	Brattleboro	WAG	Brattleboro
Blood count						
neutrophils	14.8±4.1	14.6±1.5	9.6±1.9	30.8±3.8*	19.2±1.8	39.4±3.2*
monocytes	7.8±2.1	11.4±2.6	5.6±1.3	11.0±0.9**	13.8±3.7	10.6±2.9
lymphocytes	71.3±2.1	71.2±2.6	79.4±2.4	49.8±4.4*	58.4±4.9	44.4±2.8**
eosinophils	5.3±1.4	2.0±0.9	5.2±1.1	8.4±0.9	10.6±1.8	5.0±1.1***
Functional activity						
neutrophils	—	—	—	—	7.1±0.8	4.9±1.0
monocytes (macrophages)	—	—	—	—	42.9±2.7	26.6±3.5**
Weight indexes of organs						
thymus	2.8±0.6	2.7±0.2	1.0±0.2	0.7±0.3	1.0±0.1	0.3±0.1*
spleen	8.6±1.5	5.7±0.8	3.7±0.1	2.5±0.2*	4.7±0.4	1.9±0.1*
APC						
primary immune response	0.5±0.1	0.6±0.2	6.9±1.2	8.1±2.1	12.1±2.5	2.6±0.5**
secondary immune response	6.2±1.9	2.5±1.0	2.7±0.1	2.1±0.2	14.9±2.6	3.4±0.6**

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to WAG rats.

WAG rats. These differences in blood count probably reflect specific features of cell migration from the bone marrow, regeneration, and functional activity. These properties play an important role in nonspecific and specific immune reactions. The intensity of phagocytosis did not differ in Brattleboro and WAG rats. Treatment with TNF-α increased the number of phagocytizing macrophages only in WAG rats. Therefore, the increase in the total count of blood monocytes in the absence of vasopressin is accompanied by a decrease in the sensitivity to stimulators of phagocytosis. Published data show that vasopressin increases phagocytic activity of macrophages in intact rats and restores impaired function in Brattleboro rats [12,15]. The NBT test revealed no interstrain differences in activity of neutrophils.

The thymus is a central organ of the immune system. We found that age-related involution of the thymus in Brattleboro rats proceeds more rapidly than in WAG rats. Maximum differences were observed between animals aging 8 months. Morphometry showed that changes in the thymus develop in the medullar layer and primarily concern selection of T lymphocytes. This process plays a key role in functional activity of the immune system. Cooperation, differentiation, and proliferation of various cells involved in the immune response occur in the spleen [6]. The weight index of the spleen in 1-month-old Brattleboro rats was lower than in WAG rats. The differences between Brattleboro and WAG rats were observed in various periods of life. We estimated the count of APC formed in the population of nuclear splenocytes after administration of foreign antigen. During the primary immune response to single immunization the rats of both strains generated similar amounts of APC. The exception was the age of 8 months. At this period the number of APC in Brattleboro rats was much lower than in WAG rats. The age-related changes of the secondary immune response in Brattleboro rats were more pronounced than in WAG rats. The immunomodulatory effect of vasopressin can be realized via central [11] and peripheral mechanism [1,12]. These changes in the humoral response suggest that under normal conditions vasopressin synthesized in the hypothalamus plays an important role in the immunological memory. The absence of hypothalamic vasopressin cannot be compensated by its ectopic synthesis in the thymus [10].

Our results indicate that morphofunctional abnormalities of the immune system develop in Brattleboro rats with relatively persistent signs of diabetes insipidus during ontogeny. The natural age-related changes in immunoreactivity are more pronounced and more rapidly develop in mutant rats with hypothalamic deficiency of vasopressin synthesis. Brattleboro rats were characterized by a permanent decrease in the number of peripheral blood lymphocytes, increase in neutrophil count, inadequate activation of phagocytosis in macrophages, pronounced involution of the thymus and spleen, and suppression of antibody production. These changes reflect a decrease in the general and specific immune resistance associated with vasopressin deficiency.

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