GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Neuromodulatory Effects of Serotonin Antibodies on Behavioral Responses, Neurotransmitter Content in the Central Nervous System, and Ligand-Binding Activity of Central Serotonin Receptors in C57Bl/6 and BALB/c Mice

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Effects of active immunization with the serotonin-protein conjugate on open-field behavior, learning and retention of the conditioned passive avoidance response, neurotransmitter contents in brain structures, and functional activity of central serotonin receptors were studied on C57Bl/6 and BALB/c mice. Serotonin antibodies produced opposite effects on the studied parameters in genetically different mouse strains.

Key Words: *serotonin antibodies; behavior; neurotransmitters; receptors*

Correction of some congenital abnormalities in the higher nervous activity (HNA) determining negative behavioral reactions and underlying the development of psychic disorders attracts much recent attention. At the same time, pharmacological correction of genetically determined peculiarities of HNA is not sufficient to solve this medical and social problem. The idea of inverse behavioral immunoregulation [4], *i.e.* binding of biological substances regulating activity of the central nervous system (CNS), in particular, serotonin (5-HT) and dopamine (DA) or glutamate and γ-aminobutyric acid, with specific antibodies, holds great promise in this respect. Similarity of some functional properties of CNS and immune system, including long-term potentiation (enhanced antibody production) and long-term memory, provide the theoretical basis for immunocorrection of behavior. Neuroimmunomodulation of behavioral reactions with 5-HT antibodies was first demonstrated in our experiments with active immunization with protein-neurotransmitters conjugates during modeling of alcoholism, drug abuse, and Parkinsonism [3,5,6]. However, the possibility of inducing long-term changes in the behavioral profile in animals with genetically determined differences in HNA was not studied.

Here we evaluated the possibility of correcting congenital behavioral reactions in C57Bl/6 and BALB/c mice differing in open field (OF) behavior, conditioning, and response to stress [7], and studied the neurochemical processes underlying this correction.

MATERIALS AND METHODS

Experiments were performed on 48 male mice weighing 20 g. C57Bl/6 and BALB/c mice were divided into 2 groups. Experimental animals were immunized 3 times with increasing doses of 5-HT-BSA conjugate

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(2-15 mg protein/kg) at 2-week intervals. The antigen was synthesized as described previously [1]. Control mice received 0.2 ml physiological saline. Before and 1 week after each injection of the antigen or physiological saline, OF behavior was studied using an Opto-Varimex computer-assisted system (Columbus Instruments, Auto Track software). All mice were trained in a conditioned passive avoidance response (CPAR) 8 days after the second injection of preparations [8] and retention was tested 1 day after conditioning. The contents of 5-HT, DA, and their metabolites in the sensorimotor cortex were measured by liquid chromatography with electrochemical detection 1 week after the third injection of preparations [9]. Simultaneously, functional activity of central 5-HT receptors in the brain was studied by radioligand binding assay with ^{3}H-serotonin (Amersham) on membrane preparations of the brain using a Rackbeta scintillation counter (LKB-Pharmacia) [11]. Plasma antibody content was measured by enzyme-linked immunosorbent assay. The results were analyzed by Student's *t* test and Fisher φ test.

RESULTS

C57Bl/6 mice were characterized by higher locomotor activity. The numbers of rearing postures and crossed central squares were higher, while the latency of exit from the center of OF was shorter in these animals compared to BALB/c mice.

One week after the first injection of 5-HT-BSA the number of rearing postures decreased from 14.6±1.5 to 9.5±1.7 in C57Bl/6 mice ($p<0.05$), but remained unchanged in BALB/c mice.

One week after the second injection of 5-HT-BSA the number of crossed central squares in C57Bl/6 mice increased from 1.7±0.3 to 3.1±0.6 ($p<0.05$). In BALB/c mice the latency of exit from the center increased from 2.3±0.4 to 4.5±0.6 sec ($p<0.05$), which is indicative of enhanced emotional strain [2].

After the third injection of 5-HT-BSA, the latency of exit from the center increased to 6.6±1.1 sec in BALB/c mice, which coincided with maximum production of 5-HT antibodies (1:64). Behavioral characteristics of C57Bl/6 mice remained unchanged at this term.

The duration of CPAR acquisition did not differ between control C57Bl/6 and BALB/c mice (Table 1). However, parameters of CPAR retention in C57Bl/6 mice were higher than in BALB/c mice, which confirmed a higher resistance of C57Bl/6 mice to stress [7].

Immunization of C57Bl/6 mice with 5-HT-BSA had no effect on conditioning, but impaired CPAR retention, which is consistent with our previous data obtained in experiments on Wistar rats [8]. In BALB/c mice immunization impaired conditioning, but improved CPAR retention. A shorter CPAR retention in control stress-sensitive BALB/c mice compared to stress-resistant C57Bl/6 mice can be explained by the fact that conditioning is a reaction to stress [10]. Active immunization of BALB/c mice with 5-HT-BSA improved CPAR retention, which indicated that 5-HT antibodies produce an antistress effect.

In control C57Bl/6 mice the contents of 5-HT, its metabolite 5-hydroxyindole-3-acetic acid (5-HIAA), and DA in the sensorimotor cortex surpassed those in BALB/c mice (Fig. 1) and probably determined a higher locomotor activity of C57Bl/6 mice. After immunization the contents of 5-HT and 5-HIAA in the sensorimotor cortex in C57Bl/6 mice remained higher than in BALB/c mice. The content of DA in the sensorimotor cortex of C57Bl/6 mice markedly decreased after immunization, but in BALB/c mice this parameter increased. Thus, in immunized C57Bl/6 and BALB/c mice the content of DA was similar. Our results indicate that behavioral and neurochemical parameters in immunized BALB/c mice did not differ from those in C57Bl/6 mice.

These variations were accompanied by changes in ligand-binding activity of central 5-HT receptors. Im-

TABLE 1. Effect of Active Immunization with 5-HT-BSA on Conditioning and Retention of CPAR (*M*±*m*)

Parameter	C57Bl/6		BALB/c	
	control (*n*=11)	experiment (*n*=12)	control (*n*=11)	experiment (*n*=12)
Time spent in a light compartment, sec				
during learning	11.0±2.3	17.6±3.4	6.1±3.4	37.6±7.6*°°
after 1 day	170.4±9.3	129.0±16.1**	96.8±20.9°	148.9±15.2**
Number of mice with impaired CPAR retention				
abs.	2	6	9	6
%+	18	50**	75°	46

Note. *$p<0.001$ and **$p<0.05$ compared to the control; °$p<0.01$ and °°$p<0.05$ compared to C57Bl/6 mice.

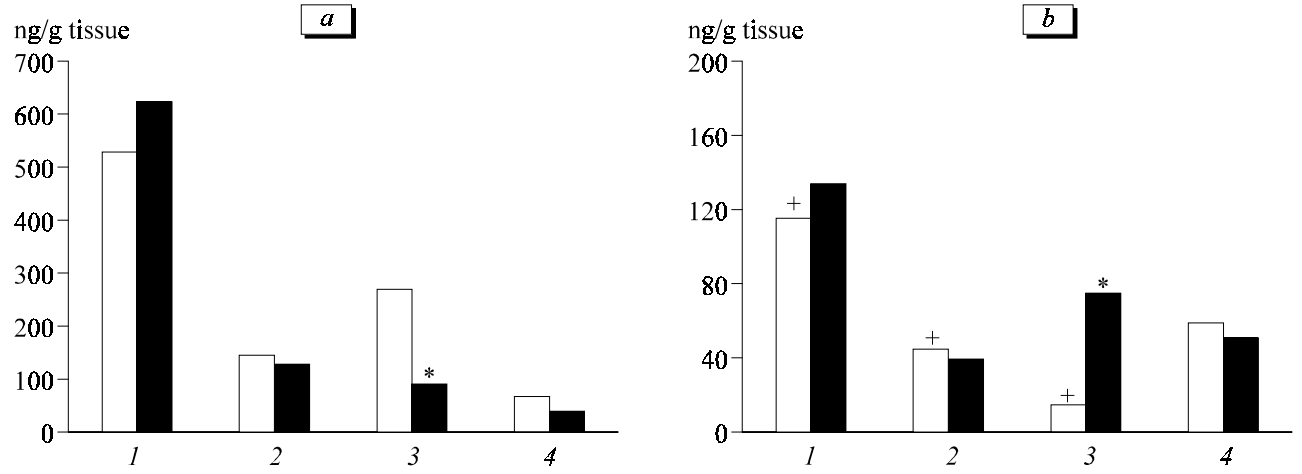

Fig. 1. Effect of active immunization with the serotonin-BSA conjugate on the content of neurotransmitters and their metabolites in the sensorimotor cortex of C57Bl/6 (*a*) and BALB/c mice (*b*): serotonin (*1*), 5-hydroxyindole-3-acetic acid (*2*), dopamine (*3*), and DOPAC (*4*). Open bars: control (physiological saline); dark bars: immunization. $p<0.05$: *compared to the control; +compared to C57Bl/6 mice.

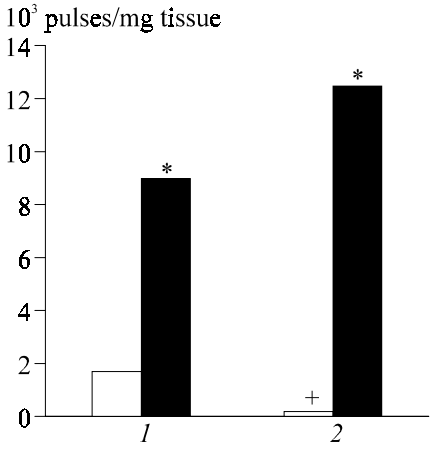

Fig. 2. Effect of active immunization with the serotonin-BSA conjugate on functional activity of central serotonin receptors in C57Bl/6 (*1*) and BALB/c mice (*2*). *$p<0.001$ compared to the control; +$p<0.01$ compared to C57Bl/6 mice.

munization with 5-HT-BSA 5.3- and 700-fold increased binding of ^{3}H-serotonin to brain membranes from C57Bl/6 and BALB/c mice, respectively (Fig. 2).

These data indicate that immunological factors associated with the formation of 5-HT antibodies produce different effects on behavioral reactions of animals and neurochemical characteristics of CNS. In C57Bl/6 mice vertical activity (rearings) decreases in the early post-immunization period, while horizontal activity (number of crossed central squares) increases in the late post-immunization period (second OF test). However, the time of CPAR retention in these animals decreased. In BALB/c mice immunization had no effect on behavioral characteristics, except for the time spent in the central zone: the increase in this parameter coincided with maximum production of 5-HT antibodies. In these animals immunization impaired conditioning, but improved CPAR retention. Thus, immunization abolished differences in behavioral characteristics of BALB/c and C57Bl/6 mice. This was probably related to changes in the content of DA in the sensorimotor cortex: this parameter decreased in C57Bl/6 mice, but increased in BALB/c mice. Pronounced differences in ligand-binding activity of central 5-HT receptors in BALB/c and C57Bl/6 mice are probably related to opposite changes in conditioning and retention of CPAR. Our results indicate that inverse immunoregulation of congenital behavior holds much promise and requires further investigations.

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