Effect of Active Immunization with Serotonin-Protein Conjugate on the Development of Experimental Parkinson's Syndrome

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Experiments on 5- and 10-month-old rats demonstrate a protective effect of active preimmunization with serotonin-protein conjugate on the development and course of the parkinsonismlike syndrome induced by intranigral injection of 1-methyl-4-phenylpyridinium$^+$. The protective effect of antiserotonin antibodies was more pronounced in younger animals. The severity of manifestations of the syndrome may depend on the level of antidopamine autoantibodies.

Key Words: *parkinsonism; serotonin; immunization; antiserotonin antibodies; antidopamine antibodies*

Insufficiency of the nigrostriatal dopaminergic system is known to play a key role in the pathogenesis of parkinsonism [8]. This system interacts intimately with the mesostriatal serotoninergic system, being in reciprocal relationship with the latter on the level of the striatum [1,9,12].

Earlier we found that in rats parkinsonismlike syndrome (PS) may be induced by intrastriatal injection of antidopamine antibodies from the serum of both immunized rabbits and patients with Parkinson's disease [4,5]. In rabbits antiserotonin antibodies injected in the striatum alleviate the symptoms of PS induced by 1-methyl-4-phenylpyridinium$^+$ (MPP$^+$) but did not prevent death [6]. These data call for experimental verification on a standard PS model in rats under conditions of active production of antiserotonin antibodies.

In light of the above, the aim of the present study was to investigate the effect of active immunization with serotonin-bovine serum albumin conjugate on the development of MPP$^+$-induced PS in rats.

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MATERIALS AND METHODS

Two experimental series were performed on 10 old (10-month-old, 470-600 g, series I) and 13 young (5-month-old, 370-450 g, series II) male Wistar rats (the indicated age corresponds to the time of PS reproduction). Each series consisted of two groups: experimental and control. At the beginning of the experiments the rats were thrice tested for 4 min in the open field for determination of the initial parameters of behavioral activity expressed as the integral behavioral index (IBI) [6]. In the successive open field tests the index of horizontal activity (ambulation) and IBI were expressed as a percentage of their initial level taken as 100%.

In each series the experimental animals were immunized with serotonin-bovine serum albumin conjugate and the controls were injected with physiological saline. The conjugate for immunization was synthesized as described elsewhere [11] using para-aminophenylalanine as the bifunctional reagent. Immunization consisted of 5 injections in ascending doses (the first injection was performed subcutaneously in complete Freund adjuvant and the oth-

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ers intraperitoneally without adjuvant). The procedure was described previously in detail [2]. PS was reproduced 14-20 days after the immunization was completed. MPP^+ (12.5 μg in a volume of 1 μl, Serva) was injected under hexenal narcosis bilaterally into the substantia nigra at a point with stereotaxic coordinates of AP=+4, L=1.5, h=8 [10]. During the subsequent 30-day observation the development and regression of PS symptoms were observed. Blood was drawn from the caudal vein before and after immunization and then additionally 2-3 times after reproduction of PS, and the content of antineurotransmitter antibodies was determined by enzyme-linked immunoassay [3].

The experimental data were processed statistically using the Student *t*, Fischer, and Spearman tests.

RESULTS

Symptoms of PS appeared the day after injection of MPP^+. The entire PS triad (oligo- or akinesia, rigidity, and tremor) was observed in all rats of both control groups. In immunized animals the entire PS triad was observed in 60 and 71% of rats in series I and II, respectively, while the other animals showed have 1-2 specific signs of PS. In both control groups the specific PS signs were more pronounced and persisted for a considerably longer time than in the experimental groups (Table 1).

Testing in the open field revealed a marked decrease in behavioral activity 1-4 days after the induction of PS (Fig. 1), motor activity being maximally reduced. On day 2 of the experiment ambulation in series I constituted 12% ($p<0.01$) and 34% ($p<0.05$) of the initial level in the control and experimental animals, respectively. In series II the respective figures were 10% ($p<0.001$) and 31% ($p<0.05$); the differences between series I and II are insignificant. Behavioral activity in the open field test was restored more rapidly in immunized animals and, above all, in young animals of series II. Toward the end of the experiment IBI returned all the way to the initial level in the experimental rats of series II and almost all the way in experimental animals of series I, while in both control groups IBI on day 30 constituted 61-62% of the initial value.

In the 3rd week after induction of PS IBI rose markedly in all immunized rats (Fig. 1), motor activity being maximally increased. In some animals ambulation surpassed the initial level 2-3 fold and their behavior resembled hyperkinesia.

In the control rats the motor activity did not return to the initial level at the end of the experiment and accounted for only 45% ($p<0.05$) and 87% ($p>0.05$) in series I and II, respectively, residual oligokinesia being more characteristic for the old animals. In some control animals tremor equivalents (nods) persisted to the end of the experiment (Table 1). In the experimental groups of series I and II the PS manifestations completely disappeared in 40 and 67% of rats, respectively.

Analysis of the rat sera for the presence of antiserotonin and antidopamine antibodies showed that before immunization antibodies to the neurotransmitters occurred in the old animals in titers of 1:16 to 1:256 but were absent in the young rats. Immunization induced the production of antibodies to neurotransmitters in rats of series II and an elevation of their titer and incidence in rats of series I. Correlation analysis showed there to be a negative relationship between behavioral activity in the open field test and the level of antidopamine antibodies in intact and immunized rats ($r=-0.89$; $p<0.001$). After injection of MPP^+ this relationship was not observed.

Analysis of the individual parameters demonstrated the most grave form of PS in control animals with the highest titers of antidopamine antibodies. The mildest symptoms followed by their

TABLE 1. Incidence (% of Animals in the Group) and Duration (Days) of the Main Manifestations of Parkinsonlike Syndrome and Pathological Signs in Rats of Different Age Immunized with Serotonin-Bovine Serum Albumin Conjugate

Pathological sign	Series I (10 months)		Series II (5 months)	
	control (n=5)	experiment (n=5)	control (n=6)	experiment (n=7)
Full akinesia	100%, 8 days	40%, 6 days	100%, 9 days	86%, 3 days
Marked oligokinesia	100%, 14 days	80%, 3 days	100%, 11 days	86%, 5-8 days
Freezing	40%, 15 days	-	-	-
Kyphosis (muscular rigidity)	100%, 20 days	60%, 14 days	100%, 16 days	86%, 10 days
Paroxysmal tremor of head and limbs	20%, 4 days	20%, 2 days	50%, 12 days	-
Myoclonia	60%, 6 days	20%, 6 days	25%, 19 days	33%, 3 days
Constant head movements	100%, 14 days	80%, 3 days	50%, 15 days	33%, 9 days
Nods	49%, 7 days	-	20%, 30 days	17%, 4 days

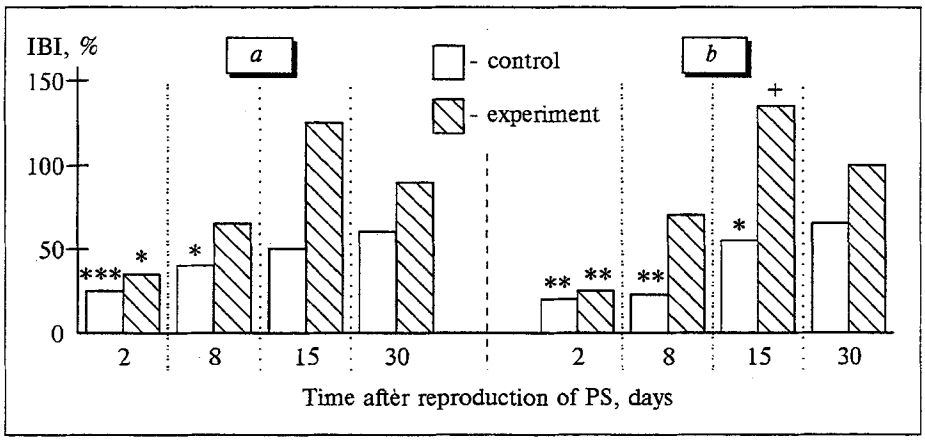

Fig. 1. Indexes of activity of 10-month-old (*a*) and 5-month-old (*b*) animals in the open field test. The initial value of the integral behavioral index (IBI) as taken as 100%. PS: parkinsonismlike syndrome; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ in comparison with the initial IBI, $^{+}p<0.001$ in comparison with the control.

complete regression were noted in experimental rats with the highest titers of antiserotonin antibodies. On the whole, the mean titers of antineurotransmitter antibodies were somewhat higher in animals of series I.

Our findings demonstrate a protective effect of active immunization with serotonin-bovine serum albumin conjugate on the development and course of Parkinson's syndrome of neurotoxic genesis in rats of different age.

Serotonin is known to inhibit the activity of dopamine-synthesizing neurons of the substantia nigra [12] and to reduce dopamine release in the striatum [13]. It may be assumed that in our experiments the antiserotonin antibodies induced by immunization suppressed the activity of the serotoninergic system of the striatum, leading to activation of the dopaminergic system and, ultimately, to alleviation of PS symptoms. In particular, the hyperkinesia in experimental rats noted in the course of the experiment may be attributed to reactivation of the extrapyramidal dopaminergic systems. This assumption may be considered to be valid in a case where antiserotonin antibodies are present in the central nervous system and it is corroborated by previous data that antibrain antibodies are able to penetrate into the brain in amounts that are minor but sufficient for the induction of specific effects [7]. Moreover, in our experiments the blood-brain barrier was mechanically damaged during PS induction.

Our experiments also demonstrated some differences in the severity and duration of parkinsonian symptoms in animals of different age groups. On the whole, the more severe form of PS was observed in older animals.

Our findings confirm previous data [6] and suggest the involvement of serotoninergic mechanisms in the pathogenesis of PS and the possibility of using antiserotonin antibodies for the correction of parkinsonian symptoms.

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