Immunocorrection of Alcohol Dependence by Immunization with a Serotonin—Homologous Protein Carrier Conjugate

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We demonstrated a possibility of inducing antibody production against serotonin in high titers in rats by active immunization with serotonin conjugated with a homologous protein carrier (rat serum albumin) and with a foreign protein carrier (BSA).

Key Words: *conjugated antigen; antibodies to serotonin; alcohol motivation*

Immunological correction of the basic neurotransmitter mechanisms underlying the formation of stable dependence on psychoactive substances, primarily ethanol and narcotics, can be realized by using antibodies to serotonin (5-HT) [2,3]. Immunization of animals (rats, mice) with 5-HT—BSA conjugate leads to suppression of alcohol dependence: decrease in alcohol consumption and suppression of the abstinent syndrome [2-4]. Induction of antibodies to 5-HT in chronically morphinized animals prevents the development of narcotic tolerance and suppresses manifestations of the abstinent syndrome [4]. Hence, clinical immunotherapy of alcoholism is possible by using 5-HT conjugate on a homologous (human serum albumin) carrier ruling out sensitization to a foreign protein. However, the antigenic characteristics and immunocorrective potentialities of this conjugate were not studied.

We studied the possibility of inducing production of antibodies to 5-HT in rats after active immunization with 5-HT conjugate with a homologous protein carrier (rat serum albumin, RSA) and the effect of this immunization on ethanol consumption under conditions of free choice.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (200-250 g). The rats were divided into 6 groups: 1) intact animals (n=4); 2) animals immunized with 5-HT—RSA conjugate (n=4); 3) immunized with 5-HT—BSA conjugate (n=4); 4) alcoholized control (n=5); 5) alcoholized rats immunized with 5-HT—RSA conjugate (n=8); and 6) alcoholized rats immunized with 5-HT—BSA conjugate (n=5).

The conjugates (5-HT—RSA and 5-HT—BSA) were synthesized in two stages [6]. Stage I consisted of protein connection to para-aminobenzoic acid in the presence of water-soluble 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide. The resultant mixture was incubated for 24 h at room temperature and then dialyzed for 5 days against distilled water. Stage II started with protein diazotization with sodium nitrite at pH 1.5, after which the solution was added to 5-HT solution (pH 8.0). All reactions were carried out at 4°C. The resultant conjugates were stirred for 1 h on ice, dialyzed for 5 days against distilled water, and stored at 4°C. The content of 5-HT in the conjugates was measured spectrophotometrically by the increment in conjugate optical density in comparison with carrier protein (5-HT absorption).

The animals were immunized with conjugate microdoses according to the protocol proposed for induction of antibodies to neuropeptides [1]. The rats received 4 injections of the conjugate over 7 weeks at 2-week intervals between the first 3 injections. The conjugate dose in complex with complete Freund adjuvant was 600 μg protein/kg for the first immunization and 800 and 1500 μg/kg in complex with incomplete adjuvant for subsequent immunizations. The mixture (0.5 ml;

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0.25 ml conjugate solution+0.25 ml adjuvant) was injected subcutaneously into 3 sites of the back. The animals were reimmunized intraperitoneally with the conjugate in a dose of 3 mg/kg in 0.5 ml saline without adjuvant 20 days after the 3rd injection. Serum antibodies were evaluated by solid-phase enzyme immunoassay with conjugate synthesized by analogous method on a foreign protein carrier (equine γ-globulin) as the test antigen. Antibody specificity was evaluated in the competitive inhibition at the counteragent concentration of 10^{-3} moles.

Craving for alcohol in groups 4-6 formed as a result of forced consumption of 15% ethanol solution for 2 weeks, followed by 2 weeks of free choice between water and ethanol. The rats received no ethanol during immunization. One week after the 3rd injection of the conjugate the animals were again offered free choice between water and 15% ethanol solution.

The data were statistically processed using Student's unpaired parametric *t* test.

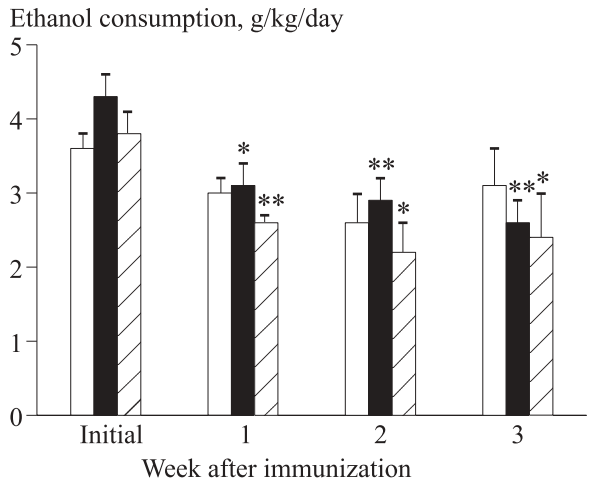

Fig. 1. Effect of active immunization with serotonin—rat serum albumin (dark bars) and serotonin—BSA (cross-hatched bars) on ethanol consumption under conditions of free choice between water and 15% ethanol solution. Light bars: control (alcoholized rats). *p<0.01, **p<0.001 compared to initial values.

RESULTS

Immunization with 5-HT—RSA antigen containing 14 5-HT molecules covalently bound to one protein molecule led to intense induction of anti-5-HT antibody production, antibody titer reached 1:8000 at the end of the experiment. In the enzyme competitive inhibition immunoassay the antibodies bound 5-HT, were inhibited by 5-HT—RSA conjugate, and cross-reacted with 5-HT—BSA conjugate. Serum titer of antibodies to 5-HT in rats immunized with 5-HT—BSA conjugate also reached 1:8000 at the end of the experiment. Hence, 5-HT—RSA antigen is characterized by pronounced immunogenic activity.

Initially alcoholized rats of experimental and control groups did not differ by ethanol consumption (Fig. 1). However, though ethanol consumption decreased in general at later terms of observation, the time course of ethanol consumption differed significantly in the groups. Immunization of rats with 5-HT—RSA and 5-HT—BSA conjugates appreciably decreased ethanol consumption in comparison with the initial level, while controls exhibited just a trend to its reduction.

Hence, we first demonstrated the possibility of inducing antibody production to 5-HT by active immunization of rats with 5-HT conjugate with homologous protein carrier. These data make us revise the common notions about inefficiency of homologous protein as a carrier. It is considered to carry no antigenic determinants recognized by T-helpers [5], but possible conformation changes in the protein molecule after its isolation are neglected. We showed that active immunization of rats with 5-HT—RSA conjugate had the same biological effect as immunization with a conjugate with a heterologous carrier.

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