Antibodies to Delta Sleep-Inducing Peptide in Ultralow Doses: Study of the Effect by Enzyme Immunoassay

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The effects of potentiated homeopathic preparations containing antibodies to delta sleep-inducing peptide in ultralow doses were studied by enzyme immunoassay. Experiments were performed with the following immunochemical reagents: antigens of delta sleep-inducing peptide conjugated to various macromolecular carriers and specific antigens. Antibodies to delta sleep-inducing peptide were synthesized in dilutions of C3, C6, C12, C50, and C200. Enzyme immunoassay showed that test preparations of antibodies in dilutions of 1:400-1:3200 produce the combined effect on immune complex formation. The proposed method holds much promise for identification of medicinal preparations in ultralow doses.

Key Words: *ultralow doses; delta sleep; potentiated substances; antibodies; enzyme immunoassay*

Recent experimental studies indicate that biologically active substances in ultralow doses (ULD), including homeopathic dilutions, modulate molecular, and cellular processes in biological systems [2,6]. The effects of biologically active substances in ULD are realized without physical transfer of individual molecules [1,7, 8]. The general principles underlying action of different homeopathically potentiated substances in ULD on various objects were evaluated [5]. The modifying effect of ULD (bipathic phenomenon) observed after combination treatment with therapeutic and potentiated medicinal preparations is of considerable practical importance. The biological effect of potentiated substances may be evaluated by clinical and biochemical parameters. However, quantities assays of potentiated substances in the solution are methodically difficult and require the use of highly sensitive methods.

We developed the method for detection of potentiated substances in ULD, including antibodies (AB) and antigens, which is based on enzyme immunoassay (EIA). Experiments were performed with AB to delta sleep-inducing peptide (AB-DSIP).

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MATERIALS AND METHODS

Conjugated antigens of DSIP were synthesized using 2 macromolecular carriers, and bovine serum albumin (BSA). Poly-4-nitrophenyl acrylate was used in EIA. AB-DSIP were obtained with BSA. Poly-4-nitrophenyl acrylate (3 mg, 0.02 mmol) was dissolved in 1 ml absolute dimethylformamide. DSIP in a final concentration of 0.001 mmol was added to the solution. Then the reaction was performed by a method for the synthesis of conjugated peptide antigens [4]. The second antigen conjugated to the protein was synthesized by the carbodiimide method using 4 mg DSIP, 30 mg BSA, 12 mg water-soluble carbodiimide, and 5 ml water. The reaction mixture was kept at 4°C for 10 h. The conjugate was isolated by gel chromatography on Sephadex G-25. AB-DSIP were obtained by immunization of rabbits [3]. The peptide-protein conjugate served as an immunogen. During solid-phase EIA the solution of DSIP conjugated to the polymer (0.5 mg/ml, 100 μl) in 0.02 M carbonate buffer was added to a 96-well plate (Nunc). The plate was maintained at 4°C for 18 h and washed 3 times with 0.05 M phosphate buffer containing 0.05% Tween 20. AB-DSIP (dilutions 1:100-1:3200) in 0.05 M phosphate buffer and 0.02% Tween 20 were added to wells. The mixture was incubated in a thermostat at 37°C for 1 h as described previously. Anti-species AB

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labeled with horseradish peroxidase were added. The plate was maintained in a thermostat at 37°C for 1 h and washed with phosphate buffer and 0.05% Tween 20. The substrate mixture containing *O*-phenylenediamine, hydrogen peroxide, and 0.05 M citrate-phosphate buffer was added. The reaction was stopped by the addition of 50 µl 15% H_2SO_4 in each well after 10-15 min. Optical density of the solution was measured on a Multiscan spectrophotometer with vertical scanning.

We used synthetic DSIP (Sigma) and conjugated antibodies against rabbit Ig labeled with horseradish peroxidase (Sigma). The results were analyzed by Student's *t* test.

RESULTS

The effects of AB-DSIP in ULD were evaluated in the model system of enzyme immunoassay, which is based on the interaction of AB and antigen immobilized on the solid phase. The results were analyzed by changes in optical density.

In series I we obtained AB-DSIP. Rabbits were immunized with the conjugate of DSIP and BSA. Immunochemical properties of antisera were studied by direct EIA. The antigen obtained after covalent binding of DSIP to polymeric matrix (DSIP-PM) was sorbed on plates to measure titer of antibodies against hapten-DSIP. Studies of rabbit antiserum obtained 3 months after the start of immunization showed that antibody titer varied from 1:200 to 1:3200. The γ-globulin fraction with highest titer of antibodies to hapten (1:3200) was isolated from the serum of the 4th rabbit and used in EIA. Specificity of AB-DSIP present in this fraction was evaluated in competitive EIA. Dermorphin, endorphin, enkephalins, and vasopressin were added to the reaction medium. These compounds (100 µg/ml-10 ng/ml) practically did not delay specific binding of AB to the antigen. These data show that the isolated serum is highly specific in relation to DSIP and may be used to evaluate the effect of potentiated antibodies (PAB) to DSIP (PAB-DSIP).

It was necessary to select the conditions optimal for the interaction between AB-DSIP and antigen on EIA plates. The optimal concentration of DSIP-PM for immobilization on the solid phase was 2 µg/ml. DSIP-PM in dilutions of 10^2-10^4 interacted with AB in the fraction from the immune antiserum. Changes of optical density in EIA were directly proportional to dilution of the serum.

In series II AB-DSIP in ULD were synthesized by the method of homeopathic potentiation. We obtained AB in dilutions of C3, C6, C12, C50, and C200 (10^{-6}, 10^{-12}, 10^{-24}, 10^{-100}, and 10^{-400} wt %, respectively). The direct interaction of AB in homeopathic dilutions with DSIP-PM immobilized on the solid phase was studied by EIA. Preparations of PAB were used instead of the antiserum. EIA showed that changes in optical density for test preparations of AB did not surpass the control. AB in ULD did not interact with the immobilized antigen.

The phenomenon of bipathy [5] observed after combination treatment with the same compound in high and potentiated doses [6] formed a scientific basis for series III. AB-DSIP in dilutions of 10^2-10^4 and equivalent volumes of AB in homeopathic doses were used in various ratios. Similar dilutions of AB-DSIP not containing the initial substance served as the control. The effect of AB in ULD on immune complex formation in the solid phase was evaluated by EIA.

After treatment with AB in dilutions of 1:400-1:3200 containing ULD of preparations, optical density decreased by 50-20% compared to the control (Table 1). Preparation C3 containing AB in various dilutions produced the combined effect on immune complex formation. AB present in preparations C12 and C6 produced these changes only in certain dilu-

TABLE 1. Optical Density of AB-DSIP Administered in Combination with the Preparation in Homeopathic Doses in EIA (*M*±*m*, optical density U)

Preparation	Dilutions of AB-DSIP							
	100	200	400	800	1600	3200	6400	12800
Control	1.49	1.22	0.88	0.53	0.31	0.23	0.12	0.04
C3	1.42	0.86	0.42*	0.24*	0.16*	0.10*	0.07	0.03
C6	1.38	1.06	0.62	0.27*	0.19	0.09*	0.08	0.02
C12	1.35	0.91	0.57	0.28*	0.16*	0.10*	0.07	0.03
C50	1.42	1.04	0.60	0.33	0.21	0.17	0.14	0.07
C200	1.30	0.91	0.62	0.35	0.19	0.16	0.12	0.06

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

tions. AB in C50 and C200 tended to modulate the interaction between AB-DSIP and antigen in EIA. However, these changes were less significant. It should be emphasized that various PAB (C3, C6, C12, C50, and C200) containing AB only in a certain dilution produced the dose-dependent effect. In higher dilution of AB (1:800) preparations C3, C6, and C12 produced the combined effect in EIA.

Our results indicate that AB in ULD and standard concentrations produced the polymodal effect in EIA. These data form the basis for evaluation of molecular mechanisms underlying the influence of AB in ULD. The proposed method holds much promise for the synthesis of new medicinal preparations and identification of substances in ULD. This problem require further investigations.

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