Detection of Antibodies to Opioid and Glutamate Receptors by Latex Agglutination and Enzyme Immunoassay

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We studied adsorption capacity of 5 latexes to synthetic peptide fragments of μ- and δ-opioid receptors and to GluR1 and NR2A subunits of glutamate receptor. Levels of autoantibodies to opioid receptors in the latex agglutination test and enzyme immunoassay were in good correlation. The level of autoantibodies to opioid receptors measured by these methods was increased in patients with opium narcomania, while the content of autoantibodies to the glutamate receptor subunits was increased in epileptics.

Key Words: *latex agglutination test; enzyme immunoassay; autoantibodies*

Increased level of antibodies to receptor proteins of nervous tissue in neurological diseases is a known fact. The content of autoantibodies to GluR1 and GluR3 glutamate receptor subunits increases in paroxysmal conditions of the brain, content of autoantibodies to NR2A glutamate receptor subunit increases in cerebral circulation disorders, and the levels of autoantibodies to μ- and δ-opioid receptors increase in opium narcomania [4,5,10,12]. These changes are presumably due to specific involvement of certain receptor proteins in the pathological process and their intense destruction and production of antibodies to the receptor fragments. Accumulation of antibodies to antigenic determinants in the blood can be detected by immunological methods. Enzyme immunoassay of autoantibodies (in particular, IgG) to glutamate and opioid receptors was developed at the Institute of Brain Research. This method can be used in laboratory diagnosis of neurological diseases and opium dependence [5]. However, latex agglutination test (LAT) is a simpler method widely used in laboratory practice for antibody assay [1,2]. This method requires no expensive equipment, is easily reproducible, and simple. Immunoreagents binding to latex particles are used for creating agglutination latex systems. Proteins and synthetic peptide oligomers (antigenic determinants) play the key role among the immunoreagents. Antigen-sensitized latex can adsorb antibodies from biological fluids [6]. We used synthetic peptide fragments corresponding to amino acid sequences of glutamate and opioid receptors as sensitizing agents.

The aim of this study was to evaluate the possibility of detecting autoantibodies to opioid and glutamate receptors in the sera of patients with opium narcomania and epilepsy by LAT and to compare its efficiency with EIA.

MATERIALS AND METHODS

Antibodies to synthetic peptide fragments of μ- and δ-opioid receptors and glutamate receptor GluR1 and NR2A subunits were evaluated by LAT and EIA in the blood of 96 individuals. Synthetic peptides consisting of 15-20 amino acids corresponding to amino acid sequences of conservative cytoplasmic domain common for human μ- and δ-opioid receptors, extracellular part of AMPA glutamate receptor GluR1 subunit and NMDA glutamate receptor NR2A subunit were obtained by solid-phase synthesis (Genemed Biotechnologies, Inc.). The purity of the peptides varied from 90 to 98%.

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The study was carried out in 23 patients suffering from opium narcomania, 26 epileptics, and 28 donors using no narcotics or sedative drugs (control group). All individuals aged 16-35 years. The blood was collected from opium addicts during 1 months after narcotic discontinuation. The serum was stored at -70°C before analysis for 6 months.

Several series of monodispersed latexes based on polystyrene (PS) and polymethylmethacrylate (PMMA) with carboxyl groups and polystyrene copolymers with acrolein (PSAC) and PMMA with acrolein (PMMA AC) synthesized at Institute of High Molecular Compounds [7-9] were selected for preparation of latex diagnostic systems.

The synthetic peptide fragments of μ- and δ-opioid receptors and glutamate receptor GluR1 and NR2A subunits were immobilized on latex particles as follows. Before sensitization latex particles were washed twice in phosphate buffer (pH 8.0), their concentration was brought to 10 mg/ml, and they were mixed with the antigen. The sensitizing dose of the antigen was 10 μg/ml latex. The mixture was incubated for 1.5 h at 37°C, the particles were washed with phosphate buffer and incubated in glycin buffer (pH 7.8) for 1 h for inactivation of free functional groups, after which the latex particles were transferred into phosphate buffer. The working concentration of polymer for latex test in plates was 0.5 mg/ml. The sensitivity of latex diagnostic agents was evaluated in LAT. The final titer of antibodies with the standard serum, depending on the latex type, was determined in studies of the sensitivity of latex diagnostic agents with the antigens. The reaction was evaluated visually by the 4-plus system. The maximum dilution of the serum giving 4+ latex agglutination was taken for the serum titer. Complete absence of agglutination was observed in titration of sera with control latexes (not modified by antigens).

Enzyme immunoassay of autoantibodies to synthetic peptide fragments of opioid and glutamate receptors was carried out on Biohit immunological plates with high adsorption capacity. Synthetic peptide fragment of μ- and δ-opioid receptor was applied in a dose of 0.5 μg/well. Before the analysis the plates were washed in phosphate buffer containing 4×10^{-4} M Twin-20 (200 μl/well for 10 min) and then incubated with 100 μl sera for 1 h on a shaker. The sera were diluted 1:50 with phosphate buffer. After triple washout with phosphate buffer with 4×10^{-4} M Twin-20 second antibodies to IgG (100 μl) conjugated with horseradish peroxidase were applied and incubated for 1 h on a shaker. The optimum dilution of second antibodies was determined experimentally; usually it was 1:10,000-1:30,000. After incubation the plate was washed with phosphate buffer with 4×10^{-4} M Twin-20 (200 μl, 10 min×3 times). For staining development, 100 μl orthophenylenediamine solution (0.5 mg/ml) in 0.05 M phosphate citrate buffer (pH 5.0) with 4×10^{-3} M H_2O_2 was applied and incubated for 20 min in darkness. Staining development was stopped by adding 30 μl 50% H_2SO_4. Optical density was measured at λ=490 nm on a Dinatech multichannel spectrophotometer.

The relationship between the parameters was evaluated using Pierson's correlations coefficient, the statistical significance of differences between the groups was evaluated using Student's *t* test.

RESULTS

Latex surface hydrophoby and type of functional groups are essential for optimal adsorption of peptide fragments on the surface of latex particles, and therefore 5 types of monodispersed latexes with different diameters of particles: PSAC (0.61 μ), PMMAAC (1.02 μ), PMMA (0.72 μ), and PS (1.00 and 0.84 μ) were used in the study.

Studies of polymeric suspensions with standard serum detected the optimal type of latex and functional groups ensuring high sensitivity of the test, evaluated by the maximum antibody titer detected in LAT with the standard serum. Serological activity in LAT with the antigen of synthetic fragments of μ- and δ-opioid receptors and different latexes was maximum (1:8 titer) for PS particles with carboxyl groups (1 μ in diameter), while a less hydrophobic PMMAAC latex gave 1:4 titer. The maximum titers for GluR1 and NR2A glutamate receptor subunits (1:8) were detected with PMMAAC copolymer carrying polymeric chains on its surface (these chains are enriched with hydrophilic comonomer acrolein containing aldehyde groups capable of chemical binding of the peptide antigens as amino components under conditions of adsorption) [7]. Latex on the base of PMMA (the least hydrophobic polymer carrying only carboxyl groups on its surface) showed no serological activity with any of the antigens. The best results were obtained in LAT with the largest latexes particles (about 1 μ in diameter), because they formed larger agglutinates and hence, the results were easier to evaluate visually.

The group of patients with opium narcomania was characterized by high incidence of increased level of antibodies to opioid receptors (antibody titers ≥1:16, not observed normally, was considered as increased; Table 1). Antibodies in titers of 1:16 and higher were detected in 71% patients with opium narcomania. The mean titer in this group was 1.4 times higher than in epileptics and 2.8 times higher than in donors.

Higher incidence of antibody titers ≥1:16 in opium addicts in comparison with epileptics attests to specificity of the detected changes in the levels of antibodies to μ- and δ-opioid receptors in these patients.

TABLE 1. Level of Autoantibodies (AA) to Opioid Receptors Detected by LAT

Group	Antibody titer to opioid receptors ($-\log_2$), $M\pm m$	Distribution by AA level			
		<1:16		≥1:16	
		abs.	%	abs.	%
Donors (n=28)	1.32±0.15	28	100	0	0
Epilepsy (n=26)	2.65±0.33*	18	69.2	8	30.8
Opium narcomania (n=21)	3.81±0.36*+	6	29.0	15	71.0

Note. *p<0.001 compared to donors; +p<0.05 compared to epileptics.

TABLE 2. Level of AA to GluR1 Glutamate Receptor Subunit Detected by LAT

Group	Titer of antibodies to GluR1 glutamate receptor subunit ($-\log_2$), $M\pm m$	Distribution by AA level			
		<1:32		≥1:32	
		abs.	%	abs.	%
Donors (n=26)	3.38±0.19	24	92.3	2	7.7
Opium narcomania (n=23)	3.34±0.26	20	87.0	3	13.0
Epilepsy (n=16)	4.93±0.26*+	5	31.3	11.0	68.7

Note. *p<0.001 compared to donors, +p<0.001 compared to opium narcomania.

TABLE 3. Level of AA to NR2A Glutamate Receptor Subunit, Detected by LAT

Group	Titer of antibodies to NR2A glutamate receptor subunit ($-\log_2$), $M\pm m$	Distribution by AA level			
		≤1:16		>1:16	
		abs.	%	abs.	%
Donors (n=28)	2.63±0.92	25	89.3	3	10.7
Opium narcomania (n=23)	3.34±0.25	21	91.3	2	8.7
Epilepsy (n=21)	3.23±0.2	19	90.5	2	9.5

The level of antibodies to μ- and δ-opioid receptors in the patients with opium narcomania was measured by EIA. The level of antibodies (0.39 arb. opt. dens. units) corresponding to the increase in optical density by 2 standard deviations from the mean value in the control group, was taken for the upper boundary of the normal. Antibody levels were elevated in 69% patients. The levels of autoantibodies to μ- and δ-antigens (antibody titer ($-\log_2$) $M\pm m$) detected by EIA and LAT in normal subjects (n=28) were 0.291±0.032 and 1.32±0.81, respectively, *vs.* 0.441±0.123 and 5.20±1.98 in the group of patients with opium narcomania (n=42). Antibody levels in patients with opium narcomania, measured by LAT, and EIA, were in significant correlation (r=0.77; p<0.05). High levels of antibodies detected by LAT in narcomaniacs were not always confirmed by EIA. The difference can be explained as follows: EIA measures IgG to the studied antigens, while LAT also detects antibodies of other main isotypes (M and A). Our comparative study showed significant differences in antibody levels in patients with opium narcomania and donors, which reflects induction of antibodies to opioid receptor in chronic opium intoxication. Antibodies to opioid receptors in the blood of narcotic users can serve as an important diagnostic sign. The advantage of this method in comparison with toxicological expert evaluation of biological fluids, indicating only a single intake of narcotics, is the possibility of detecting antibodies to opioid receptor in remote period after the last intake of the narcotic, this indicating chronic opium intoxication [3].

Antibodies to the glutamate receptor GluR1 and NR2A subunits in the sera of patients with opium

narcomania and epilepsy and donors were measured by LAT (Tables 2 and 3). The titer of antibodies to GluR1 subunits ≥1:32 was considered elevated, because this titer was detected in only 7.7% donors. The levels of antibodies to the glutamate receptor NR2A subunit in the sera of patients with opium narcomania and epilepsy and in donors virtually did not differ. These data are in line with previous reports increased level of antibodies to the glutamate receptor GluR1 subunits in epileptics correlating with the disease duration and severity [11].

Hence, LAT can be regarded as a simple and reliable method for semiquantitative evaluation of autoantibodies to nervous tissue receptor proteins; along with EIA, this method can be used for the diagnosis of many neurological and narcological diseases.

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