The existence of Ca-dependent and Ca-independent mechanisms of cellular adhesion in vivo has been suggested [9]. Whatever the mechanisms of cellular adhesion in vivo, especially during embryogenesis and regeneration, a leading role in cellular aggregation preceding adhesion is played by HUA, PCKS, and other proteoglycans existing in the form of salts, including their potassium and calcium salts [9]. In this connection the possibility cannot be ruled out that the effect of Ca^{++} on cellular aggregation and adhesion is effected to some degree through HUA and PCKS.

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PREPARATION OF PORCINE MB CREATINE PHOSPHOKINASE HETERODIMER AND STUDY OF ITS BINDING WITH POLYCLONAL ANTIBODIES

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KEY WORDS: creatine phosphokinase heterodimer; polyclonal antibodies; conjugates with peroxidase.

One stage in the investigation of the structural—immunological properties of enzymes is to determine the degree of affinity of antibodies obtained to different molecular forms of an enzyme [3, 5].

The aim of this investigation was to study these properties of porcine creatine phosphokinase, which is a dimer consisting of M and B subunits, which give rise to three corresponding molecular forms of the enzyme: MM, MB, and BB.

EXPERIMENTAL METHOD

The protein content at all stages of isolation and purification of antigens and immunoglobulins and of synthesis of conjugates was determined by Lowry's method [2]. The immunoprecipitation test was carried out by Ouchterlony's method [4]. M creatine phosphokinase was isolated from porcine skeletal muscles. BB creatine phosphokinase was isolated from porcine brain by double ion-exchange chromatography on DEAE-cellulose followed by chromatography on hydroxyapatite. Rabbits were immunized 6 times at intervals of 3 weeks: with 1 mg of enzyme in 1.5 ml of 0.05 M Tris HCl buffer (pH 8.9) with Freund's complete adjuvant. Conjugates of antibodies and antigens were synthesized with horseradish peroxidase [1, 7].

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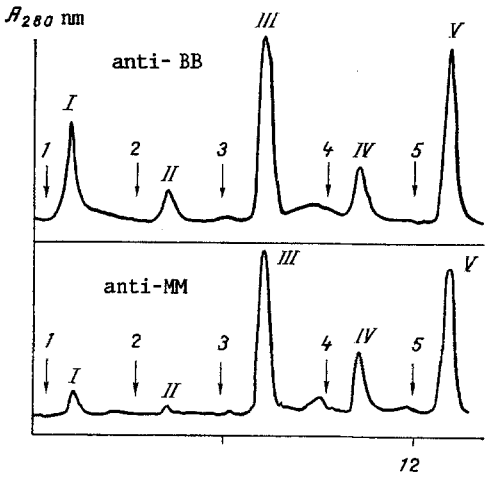

Fig. 1. Fractionation of antibodies against MM- and BB-isozymes of creatine phosphokinase on DEAE-cellulose A-52. Column 2.5 × 30 cm. Rate of elution 24 ml/h. Stepwise gradient: 1) 5 mM K-phosphate buffer (KPB), pH 8.0; 2) 10 mM KPB, pH 6.35; 3) 40 mM KPB, pH 6.0; 4) 0.1 M KPB, pH 5.8; 5) 0.3 M KPB, pH 5.4.

EXPERIMENTAL RESULTS

To determine whether creatine phosphokinase can bind simultaneously with anti-M- and anti-B-antibodies, the hybrid MB isozyme was synthesized and isolated. For this purpose the MM- and BB-isozymes, kept after isolation in 60% ammonium sulfate in the form of a finely crystalline suspension, was dialyzed at 2-4°C against 0.01 M Tris-HCl buffer (pH 8.5). To prevent oxidation and inactivation, dithiothreitol was added up to a 5 mM concentration. The MM- and BB-isozymes were mixed in equimolar proportions under dissociating conditions in the presence of 3 M guanidine chloride and incubated at 4°C for 2 h. After removal of the dissociating agent by dialysis, preparative electrophoresis was carried out in 7% polyacrylamide gel. Zones corresponding to the MB-isozyme in their electrophoretic mobility and activity were cut out of the gel. The enzyme was eluted and used both to obtain conjugates and for testing by ELISA.

By fractionation of polyclonal rabbit antibodies against porcine creatine phosphokinase on DEAE-cellulose A-52, five fractions of immunoglobulins were isolated (Fig. 1). Fraction III (IgG_1), as the most active in the precipitation test, was used to obtain conjugates of the immunoglobulins with peroxidase.

The use of different versions of immunoenzyme analysis revealed low affinity of anti-BB antibodies, and it was concluded that the use of anti-BB conjugates in the sandwich method of determination of MB-isozymes is ineffective. Covalent binding with peroxidase reduces by an order of magnitude the binding constant of the antibodies. Meanwhile testing with the aid of peroxidase conjugates of goat antibodies against rabbit immunoglobulins showed that peroxidase activity is a linear function of the quantity of the corresponding immunoglobulin, bound with the MM-isozyme, within the range from 10 to 900 ng/ml.

Thus to create a system in which the heterodimer isozyme of creatine phosphokinase reacts simultaneously with anti-M- and anti-B-immunoglobulins, it is essential to have antibodies of higher affinity than those obtained by immunization of a rabbit with native porcine antigen. One possible source of such immunoglobulins could be, for example, monoclonal antibodies or antibodies obtained against antigens with enhanced immunogenicity, and isolated with the aid of immunosorbents.

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BIPHASIC ACTION OF IMMUNOGLOBULINS ISOLATED FROM BLOOD OF MYASTHENIA PATIENTS ON MICE

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KEY WORDS: myasthenia; immunoglobulins; phases of physiological action.

The pathogenesis of myasthenia is nowadays regarded as a rule from the standpoint of the autoimmune hypothesis. According to this hypothesis the appearance of myasthenic disorders is the result of blockade of the nicotinic acetylcholine receptor (NAR) of the myoneural synapse by autoantibodies specific to it which appear in the patient's body. It is considered that these autoantibodies belong to the IgG class [4, 5]. However, according to some authorities, the absence of correlation between the titer of specific anti-NAR autoantibodies of the IgG class and the severity of the disease is not in agreement with these views [3, 6, 8].

To study the effect of different classes of immunoglobulins on the formation of the myasthenic syndrome, the character of development of myasthenia was studied on a passive transfer model [7].

EXPERIMENTAL METHOD

Blood plasma from a patient with severe myasthenia, obtained during a plasma exchange operation, and blood plasma from a healthy donor were used. The immunoglobulin fraction was isolated from this material by precipitation with ammonium sulfate. Preparations of IgG and IgM were isolated from the fraction thus obtained, by ion-exchange chromatography on a column with DEAE-Sephadex A-50. The IgM preparation was further purified by gel-chromatography on a column with Sephadex G-200 [2]. Sucrose was added to the immunoglobulin preparations up to a final concentration of 5% and the samples were poured into ampuls in a volume of 2 ml, lyophilized, and sealed. The preparations were kept at 4°C before use in the experiments. Before being injected into the animals the preparations were dissolved in distilled water, the volume of water taken being sufficient to ensure that the dose of the preparation injected into the mouse was contained in a volume of 0.1-0.5 ml.

The experiments were carried out on female C57 mice weighing 19 ± 3 g. Preparations of immunoglobulins were injected intraperitoneally in single doses of 2, 4, 6, 8, and 10 mg per mouse. Each dose was given to two (IgM) or five (IgG) mice. Altogether 35 mice receiving immunoglobulins from blood plasma of the myasthenia patient and the same number of animals receiving immunoglobulins from plasma from a healthy blood donor were used in the experiments. No immunodepressants were given.

To evaluate the action of the immunoglobulin preparations on the neuromuscular synapse, a test with neostigmine was used. The anticholinesterase agent was injected intraperitoneally into the mice in a dose of 0.2 ml of a solution containing 5 μg of the drug in 1 ml. The motor activity of the mice was estimated qualitatively or by the test based on the ability of the mouse to lift itself up by its tail when held with forceps at a distance of 1.5-2 cm from the tip (Fig. 1d, e). If the mice could perform the test in under 60 sec, the time of test performance was estimated. In this case the data were subjected to statistical analysis by

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