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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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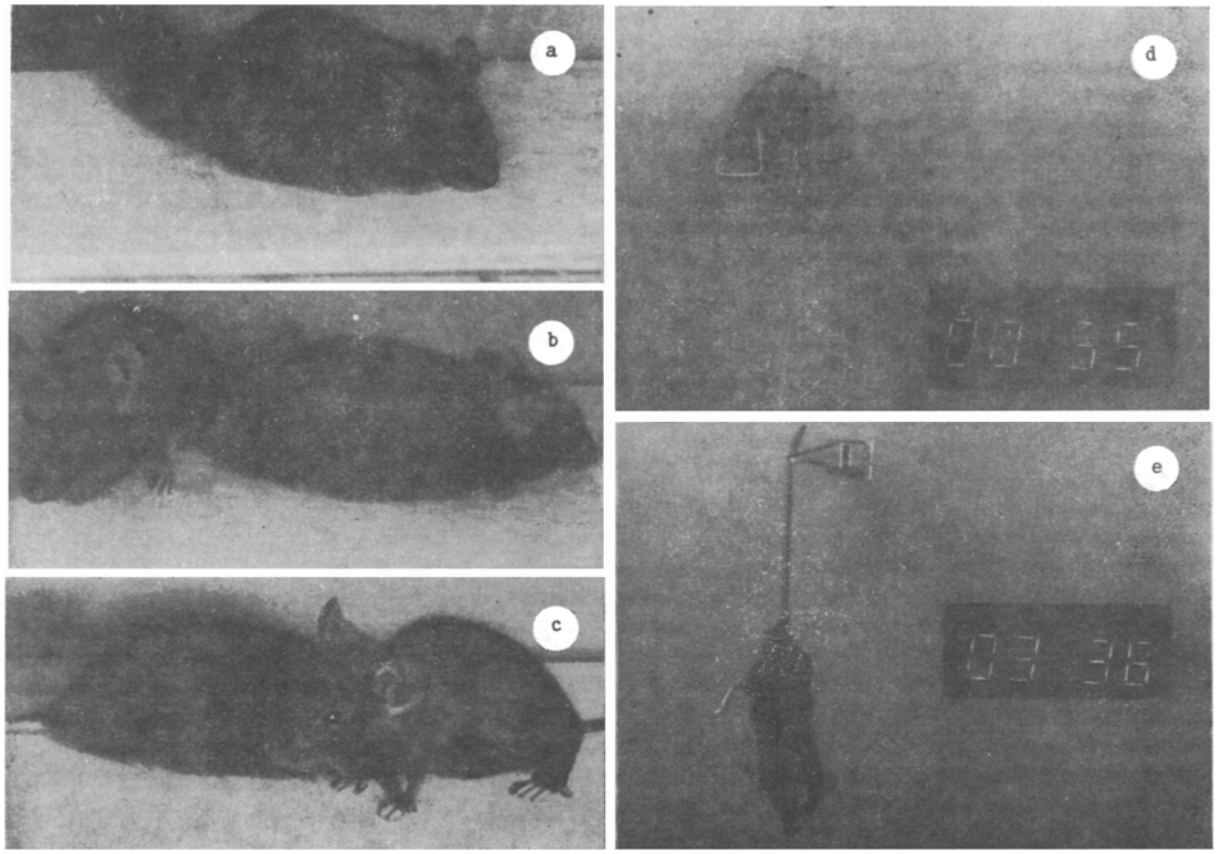

Fig. 1. Mice after injection of immunoglobulin preparations from blood of myasthenia patient. a, b, c) Mice 3 h after injection of IgM preparation in a dose of 10 mg (mouse in center of frame) and IgG preparation in the same dose (b — mouse on the left; c — mouse on the right); d and e) mouse performing test of lifting itself up by its tail 3 weeks after injection of immunoglobulin preparations. Time after beginning of performance of test (in minutes and seconds) indicated on display.

Student's test [1]. If the test performance by the mice was irregular, the frequency of its performance by a group of mice (10-12 animals) was estimated in a series of attempts, each lasting 15 sec, with an interval of 2-3 min between attempts. In this case, the chi-square method was used for statistical analysis of the results [1].

EXPERIMENTAL RESULTS

After chromatography the immunoglobulin preparations were obtained as solutions with protein concentrations of 2-2.5% (IgG) and 0.6-0.7% (IgM). The yield from 150 ml plasma was about 0.1 g for the IgM.

In mice receiving the IgG preparation from blood of the myasthenia patient, no marked disturbances of motor activity were observed in the first week of the experiment. In mice receiving the IgM preparation from the same material, a myasthenic syndrome was observed as early as in the first few hours after injection of the preparation; moreover, disturbances of movement were more marked in animals receiving the preparation in doses of 6, 8, and 10 mg (Fig. 1). The neostigmine test was positive — temporary partial recovery of mobility was observed 1-2 min after injection of neostigmine; its action continued for 2-3 min. On the 2nd day of the experiment the mobility of all the animals was restored. IgM preparations from the blood of a healthy donor caused no such disturbance of movement. Although the mice receiving the IgM preparation from the blood of the myasthenia patient regained their mobility, and mice receiving IgG preparations from the same material had no marked myasthenic disturbances, in all these animals, unlike mice receiving IgM and IgG from the healthy blood donor, there was some reduction of mobility. To assess this state objectively, the test of lifting the mouse by its tail was used. Control animals performed the test in 0.5-4.7 sec ($p < 0.01$). Mice receiving

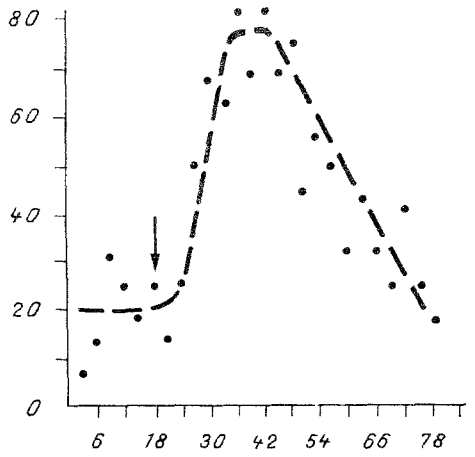

Fig. 2. Effect of neostigmine on frequency of performance of lifting the mouse by its tail test by mice of group with late myasthenic disturbances. Neostigmine injected after 18 min (arrow); each dot denotes results of 16 tests in three experiments during the 5th week of the investigation. Abscissa, time (in min); ordinate, frequency of performance of test in group (in %).

the IgM preparation from the blood of the myasthenia patient could not perform this test the next day, whatever the dose received (Fig. 1). The ability of all these animals to perform this test was restored later. Mice receiving the IgG preparation from the blood of the myasthenia patient performed the test somewhat worse in the first week: the scatter of the test performance time in each group of animals became wider, although no difference was found between the groups. For example, on the 5th day of the experiment mice receiving the IgG preparation (10 mg) performed the test in 2.1-13.1 sec ($p < 0.01$).

A few days after injection of the immunoglobulin from the blood of the myasthenia patient, some mice began to perform the test of lifting the mouse by its tail, but not at all attempts. In the second week of the experiment, a group of 10 to 12 mice was formed from these animals, containing nearly all the animals receiving the immunoglobulin preparation in a dose of over 6 mg. Some animals of this group ceased completely to perform the test. These animals gradually died. Altogether during 7 weeks of the experiment, of 35 animals receiving immunoglobulins from the myasthenia patient, seven died; of this number five received IgG in doses of 6 mg (three mice) 8 and 10 mg (one mouse each), and two received IgM in doses of 2 and 10 mg.

To assess the effect of neostigmine on mice which could no longer perform the lifting by its tail test at all attempts, the method of evaluation of the frequency of test performance in a group of 10-12 animals at equal time intervals before and after injection of the anticholinesterase drug was used. The results of determination of the frequency of test performance on the 19th, 20th, and 22nd days of the investigation are given in Fig. 2. Each dot on the graph is the average value of performance of the test by a group of mice in three experiments, in percent, obtained during the study of the effect of neostigmine on the frequency of test performance. In the patient from the 10th to the 40th minute, marked facilitation of test performance was observed. The significance of the effect of neostigmine 20 min after its injection exceeded 90%.

It can thus be concluded from the experimental results that autoantibodies of the IgM class play an essential role in the formation of myasthenic disturbances of autoimmune nature. The negative data obtained previously [7] in a study of the role of IgM in the development of myasthenic disorders can probably be attributed to the fact that the authors cited used a less effective method of isolating immunoglobulins and smaller doses of IgM in experiments with a model of passive transfer of the myasthenic syndrome. A biphasic action of immunoglobulins from the blood of a patient with severe myasthenia, discovered in this investigation, indicates that attack on NAR of the myoneural synapse is not the only cause of the appearance of symptoms linked with the autoimmune component of the pathogenesis of the disease. Autoantibodies of the IgM class are probably more effective blockers of NAR, as is confirmed by the time course of the action of neostigmine. However, the acute effect of blocking NAR is of

short duration and does not lead to a lethal outcome. Meanwhile the late effect of injection of heterologous autoantibodies is independent of the class to which they belong. This effect has a marked individual character even for inbred mice, and it is manifested several weeks after injection of antibodies without an immunodepressant. It can be tentatively suggested that the late effect of injection of heterologous autoantibodies is connected with triggering of an autoimmune process involving targets other than NAR of the myoneural junction. Animals in which this effect was exhibited often died. The time course of the action of neostigmine in this case was different from that of its action in the first phase. This also indicated that in the second phase of action of the preparation of heterologous autoantibodies in the passive transfer model, targets other than NAR of the myoneural junction are involved.

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HUMAN CHORIONIC PREALBUMIN: IDENTIFICATION, PHYSICOCHEMICAL PROPERTIES AND ITS DETECTION IN BLOOD SERUM IN TROPHOBLASTIC DISEASES

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KEY WORDS: human chorionic prealbumin; immunodiffusion analysis; trophoblastic diseases.

Investigations of hormones produced by the human placenta began in the 1930s-1950s after the discovery of chorionic gonadotropin [2] and of placental lactogen [4]. However, a new stage in the study of placental proteins began with the discovery of trophoblastic γ-globulin [1, 5], which was followed by the discovery of a whole group of "specific" placental proteins, possessing neither hormonal nor enzymic activity [3].

This paper gives information about human chorionic prealbumin (CPA), some of its physicochemical properties, and the results of its immunodiffusion analysis in patients' tissues and blood sera.

EXPERIMENTAL METHOD

Antiserum to CPA was prepared by immunizing rabbits with semipurified preparations of CPA isolated from extracts of the chorion obtained during therapeutic abortion at the 8th-12th week of pregnancy. The semipurified preparation was obtained by freezing and thawing chorionic extracts 3 times, with the addition of 1% Triton X-100, followed by dialysis against 0.15 M sodium chloride in 20 mM Tris-HCl, pH 8.0, and by salting out the fraction obtained between 40 and 70% saturation with ammonium sulfate. The fraction thus obtained was dialyzed against 20 mM Tris-HCl buffer, and subjected to adsorption chromatography on columns containing DEAE-52

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