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LOCATION OF BINDING SITES FOR COBRA NEUROTOXIN AND SERUM IMMUNOGLOBULINS FROM MYASTHENIA GRAVIS PATIENTS IN RAT MUSCLES AND BRAIN

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KEY WORDS: myoneural junctions, brain neurons, myasthenia, cobra toxin, immunohistochemistry.

Myasthenia is characterized by increased vascular fatigue and progressive muscular weakness, which can be temporarily relieved by injection of anticholinesterase drugs; in severe cases this disease causes death because of respiratory arrest [2, 3]. According to the generally accepted views, the immediate cause of the disturbances of myasthenia is an autoimmune attack on the nicotinic cholinergic receptors (NChR) of the myoneural junction, which reduces the number of functioning NChR. However, in myasthenia disturbances of the CNS are also observed. These disturbances are connected either with the primary pathological process in the brain and are mediated by dysfunction of the thymus, or with secondary hypoxia caused

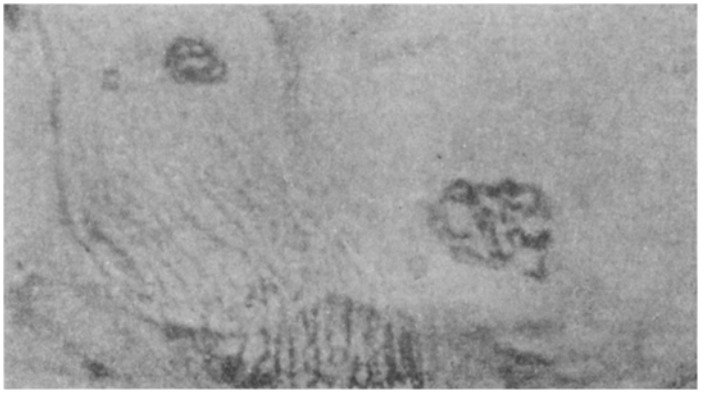

Fig. 1. Location of peroxidase reaction on incubation of sections of rat intercostal muscle with CT-HRP conjugate. Here and in Figs. 2 and 3: objective 40, ocular 10 ×.

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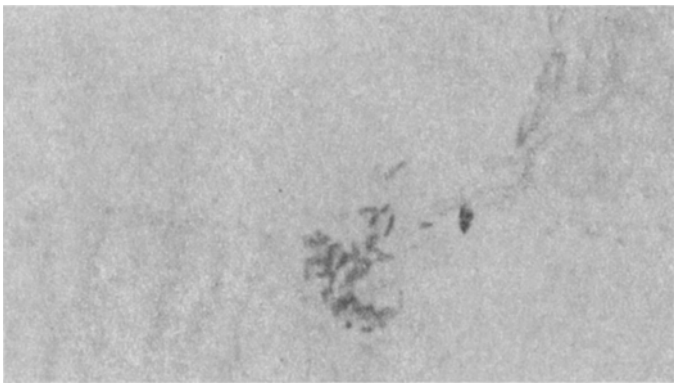

Fig. 2. Location of peroxidase reaction in myoneural junction of rat intercoastal muscle on incubation of sections with blood serum from myasthenia patients followed by detection of bound IG by the indirect immunoperoxidase method.

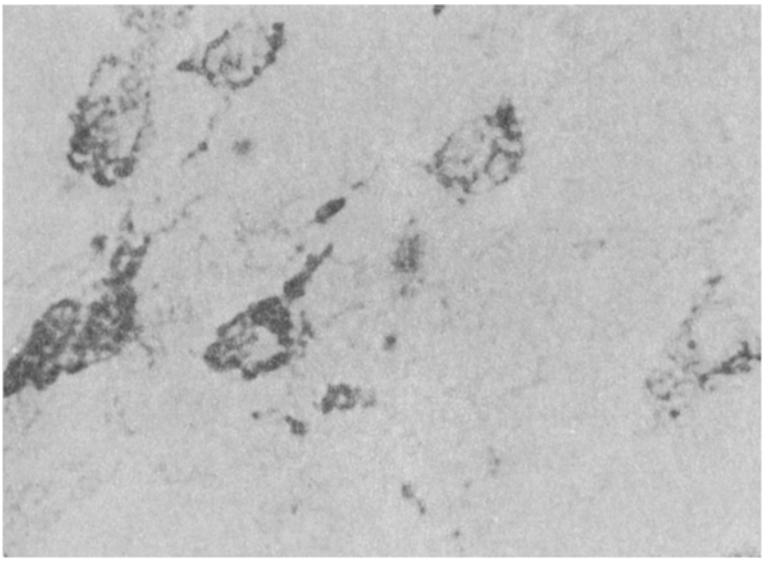

Fig. 3. Location of peroxidase reaction in neurons of claustrum of rat brain on incubation of sections with blood serum from myasthenia patients and subsequent detection of bound IG by the indirect immunoperoxidase method.

by the disturbance of respiration, and also with possible involvement of autoimmune mechanisms [2-4, 9]. The writers showed previously that NChR protein, isolated from bovine brain, when used to immunize rabbits, causes myasthenic disorders [6], and that the serum immunoglobulins (IG) of patients with myasthenia interact with central NChR [4]. The aim of this investigation was a comparative study of the location of antigenic determinants interacting with serum IG of patients with myasthenia, and binding sites of an NChR marker, namly cobra neurotoxin.

EXPERIMENTAL METHODS

The chromatographically homogeneous preparation of venom of the Central Asiatic cobra cobrotoxin 2 (CT) and horseradish peroxidase with R_Z = 3.0-3.2 were used. Human IG was isolated from serum of normal blood donors and myasthenia patients by the sulfate method and purified on DEAE-Sephadex. Antiserum against human IgG was obtained from the Gor'kii Research Institute of Vaccines and Sera. A pool of sera from 20 patients with a myasthenic syndrome, undergoing treatment at the Clinic for Nervous Diseases, S. M. Kirov Leningrad

Postgraduate Medical Institute, was used as the source of IG from patients with myasthenia. Conjugates of CT and IG with horseradish peroxidase were synthesized as described previously [1]. The conjugates were purified from unreacted components by gel-filtration chromatography. The histochemical investigation was carried out on frozen specimens from unfixed tissue of rat intercostal muscles and brain. Sections on slides were fixed in cold acetone (10 min) before incubation with the sera and conjugates. For work with sera from myasthenia patients or with IG isolated from them, the indirect immunoperoxidase method was used. Serum from normal blood donors or the IG fraction isolated from that serum served as the control. The sera were used in dilutions of 1:500-1:1500. Antisera against human IgG were used in dilutions recommended by the manufacturer. For histochemical detection of peroxidase activity the benzidine method was used [1].

EXPERIMENTAL RESULTS

The conjugate of CT with horseradish peroxidase (CT-HRP) was specifically bound with myoneural junctions of rat intercostal muscle (Fig. 1): binding was completely blocked by preliminary incubation of the sections with CT (10^{-4} g/ml) and was considerably reduced by preliminary incubation with carbachol (10^{-2} M), i.e., CT-HRP does in fact interact with muscle NChR and can be used as a marker.

The structure of the myoneural junction also could be seen in sections of the muscles both when serum from myasthenia patients and IG from that serum were used in the indirect immunohistochemical method (Fig. 2). Preliminary incubation of the sections with CT (10^{-4} g/ml) partly reduced the binding of IG from myasthenia patients with the myoneural junction. When serum from normal blood donors was used only weak diffuse peroxidase activity was observed throughout the muscle section. It is genenerally considered that serum IG from myasthenia patients bind with NChR of the myoneural junction [11], as has been shown by physiological and electron-microscopic investigations.

Biochemical and histochemical investigations using labeled α-bungarotoxin (an analog of CT) indicate that this marker can be used to isolate brain NChR [9]. However, we were unable to detect any structures in sections of rat brain tissue with the aid of CT-HRP. Meanwhile serum IG from myasthenia patients, but not serum IG from normal blood donors bound selectively with the pericellular structures of neurons in the claustrum and medullary reticular formation, as well as of individual large cells of the caudate nucleus, putamen, and gray matter of the cerebral cortex. No binding could be seen in other parts of the brain. Residue of the peroxidase reaction was localized on the cell membrane in the form of round bodies, which were particularly numerous in the region of the axon hillock (Fig. 3). Staining of the cytoplasm of nerve cells, which showed a pericellular reaction, and of the surrounding brain tissue was very weak when dilutions of the first (specific) serum (1:500-1:1500) were used. No staining of brain structures was observed in experiments with serum from normal blood donors.

This investigation showed that, besides antibodies to muscle NChR, antibodies binding specifically with antigens of certain brain neurons also participate in the pathological process responsible for the myasthenic syndrome. It can be tentatively suggested that among their antibody pool, sera from myasthenia patients contain antibodies selectively reacting only with brain antigens, and differing from antibodies which interact with NChR. Another explanation, which seems more correct to the present writers, is that antibodies to NChR of the myoneural synapse interact also with antigenic determinants of brain NChR [4]. At the same time, CT, which is a highly specialized and irreversible blocker of muscle NChR, does not form a stable complex with brain NChR, and that is why it was impossible to demonstrate the localization of brain NChR with the aid of this marker.

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SURFACE ACTIVITY OF SURFACTANT IN EXPERIMENTAL COMPRESSION ATELECTASIS

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612.212.014.1.04.462.8

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Among investigations to study the state of the lung surfactant (LS) in atelectasis [1-4, 9, 10] there have been none to study the dynamics of changes in LS at different periods of atelectasis. It is not clear how the qualitative composition of the phospholipids of LS, on which its surface-active properties depend, changes under these circumstances, or whether aeration of the lung tissue can be restored after removal of the cause of the atelectasis.

In the investigation described below a combined study was undertaken of the time course of changes in the surface activity of LS at different stages of compression atelectasis.

EXPERIMENTAL METHODS

Material for the investigation consisted of lungs of 35 guinea pigs of both sexes and weighing 150-250 g, in which compression atelectasis was modeled by the creation of hydrothorax (injection of 15-20 ml of sterile isotonic sodium chloride solution into the pleural cavity). The animals were decapitated 30 and 60 min, and 3, 12, and 24 h after injection of the solution. In each of the five groups corresponding to the above-mentioned times, six animals were killed, and five animals served as the control group. To confirm the presence of atelectasis of the lung tissue, paraffin sections of lung tissue were examined histologically, using survey stains. To investigate LS, 10% saline extracts were prepared from the lungs, from which

TABLE 1. Surface Tension of LS, Total Lipid and Phospholipid Content and Qualitative Composition of Phospholipids during Experimental Compression Atelectasis

Duration of atelectasis	ST_{min}, mN/m	CI	Content of total lipids, g/liter	Content of phospholipids		
				total, mmole/liter	phosphatidylcholine	phosphatidylethanolamine
					%	
Control	14,2±1,0	1,1±0,1	0,92±0,02	0,078±0,010	37,3±2,1	19,6±2,2
30 min	27,1±2,9	0,5±0,05	0,99±0,03	0,027±0,007	30,8±6,8	43,7±6,6
60 min	19,8±2,8	0,5±0,11	0,99±0,03	0,044±0,018	None	61,7±6,0
3 h	22,7±1,5	0,6±0,08	0,98±0,04	0,048±0,015	32,8±9,0	49,4±9,4
12 h	31,8±3,7	0,5±0,06	1,06±0,01	0,035±0,006	47,2±14,3	39,1±7,4
24 h	29,9±4,3	0,5±0,05	0,98±0,03	0,034±0,015	39,2±10,9	61,8±3,5

Legend. Results significant compared with control ($P < 0.05$) except phosphatidylcholine content and value of ST_{min} for atelectasis with a duration of 60 min ($P > 0.05$).

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