PHARMACOLOGIC PROPERTIES OF ANTIACETYLCHOLINE RECEPTOR ANTIBODIES

G. V. Burlakov

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A new discipline has appeared in immunology, namely immunopharmacology [8]. A special journal is being published [International Journal of Immunopharmacology, editors P. W. Mullen, F. Spreafico, and J. W. Hadden (the official journal of the International Society for Immunopharmacology)], devoted, in particular to the publication of papers on the pharmacologic action of antigens, antibodies, and immune complexes on cellular receptors. A particularly interesting trend in this discipline is the study of the properties of antiacetylcholine-receptor (AAChR) antibodies. The principal technical problem in this research is that of obtaining antigens (acetylcholine — ACh — receptors) in a sufficiently pure form. Some workers [1], who made use of the well know fact that skeletal muscles are greatly enriched with ACh receptors after denervation [10, 11], have obtained AAChR antibodies by immunizing rabbits with antigenic material from motor-denervated skeletal muscles of the hind limbs of BALB/*c* mice. Under these circumstances the antigenic material was isolated by the method in [9]. It was shown that treatment of mouse lymphocytes with AAChR antibodies blocks the ACh receptors of the lymphocytes, so that the latter become less sensitive to the action of the cholinergic agonist carbachol. The reaction of inhibition of immune rosette formation under the influence of carbachol was used as the test object to study the action of AAChR antibodies on mouse lymphocytes. On preliminary treatment of the lymphocytes with AAChR antibodies, no inhibitory effect of carbachol on immune rosette formation was observed. In other words, AAChR antibodies in these experiments had an atropinelike action. The question naturally arises, can an atropinelike effect of AAChR antibodies be discovered on any other biological objects more sensitive to acetylcholine and its analogs than lymphocytes.

Leeches (*Hirudo medicinalis*), which are highly sensitive to acetylcholine and have many ACh receptors per unit area of surface of their cells [3], were used to determine some of the pharmacologic properties of AAChR antibodies.

EXPERIMENTAL METHOD

Experiments were carried out on 30 leeches. The test object was a preparation of the dorsal muscle of the leech [3, 4]. The preparations were continuously aerated with oxygen. The acetylcholine chloride was obtained in ampuls from the Moscow Medical Preparations Production Combine. Rabbit serum against ACh receptors, obtained from motor-denervated skeletal muscles of the hind limbs of BALB/*c* mice, was used in dilutions of 1:10 and 1:20. The titer of antibodies in the complement fixation test (CFT) was 1/1280. The preparation was incubated with serum for between 5 and 40 min. Serum from unimmunized rabbits, and also serum from rabbits immunized with antigenic material obtained from normal (not denervated) skeletal muscles from the hind limbs of BALB/*c* mice was used as the control. These sera were used in the same dilutions and with the same incubation time.

EXPERIMENTAL RESULTS

We tested different concentrations of ACh, starting from very low (10^{-15}) and ending with very high (10^{-3}, 10^{-2}). The clearest results were obtained, however, with average concentrations (10^{-10}-10^{-8}): excessively weak concentrations gave

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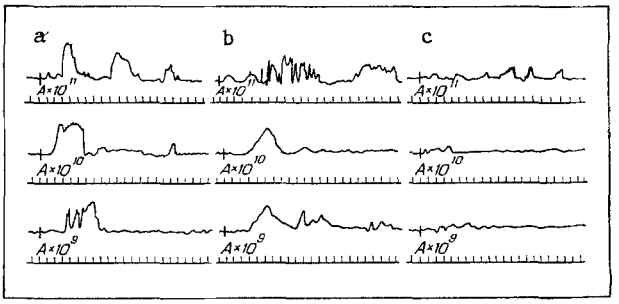

Fig. 1. Effect of AAChR antibodies on contraction of dorsal muscle of the leech under the influence of different doses of ACh. a) Before treatment with AAChR antibodies; b) after treatment with normal rabbit serum; c) after treatment with AAChR antibodies. Final dilution of sera 1:10. Incubation time of preparation with AAChR antibodies and with normal rabbit serum was 20 min. Time marker 10 sec.

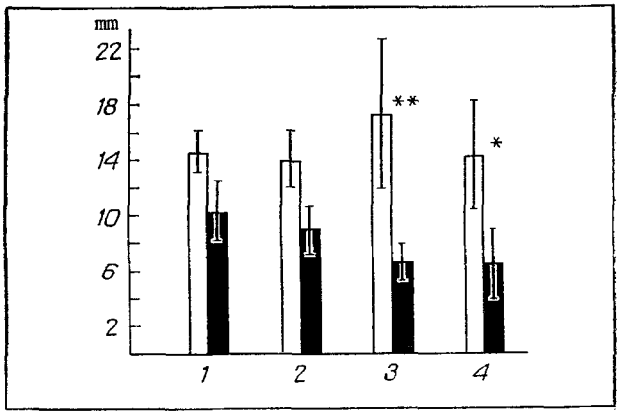

Fig. 2. Action of ACh on amplitude of contraction of dorsal muscle of leech before (unshaded columns) and after (black columns) treatment with AAChR antibodies. Time of incubation of preparation with antibodies 20 min. Final dilution of serum 1:10. $*p = 0.05$; $**p < 0.05$. Abscissa: 1) ACh concentration 10^{-11} (unshaded column, $n_1 = 15$; black column, $n_2 = 12$); 2) ACh concentration 10^{-10} ($n_1 = 7$, $n_2 = 12$), 3) ACh concentration 10^{-9} ($n_1 = 5$, $n_2 = 11$), 4) ACh concentration 10^{-8} ($n_1 = 6$, $n_2 = 11$); ordinate, amplitude of contraction of dorsal muscle of leech (in mm).

less clear results, evidently because of rapid destruction of the ACh in the medium solution containing the segment of the leech. Too high concentrations also were less effective, for they induced tachyphylaxis or led to rapid habituation of the test object to the action of ACh.

During incubation of the preparation with AAChR antibodies, obtained by immunizing rabbits with antigenic material from the motor-denervated skeletal muscles of the hind limbs of BALB/c mice, a considerable and statistically significant decline in amplitude of contraction of the dorsal muscle of the leech took place under the influence of the test doses of ACh. The results were compared with the amplitude of contraction of the muscle under the influence of the same doses of ACh before treatment of the preparation with AAChR antibodies. In the control series of experiments incubation of the preparation with normal rabbit serum and with the serum of rabbits immunized with material obtained from nondenervated mouse skeletal muscles, either did not induce any decrease, or induced a smaller decrease, in contractile activity of the dorsal muscle of the leech under the influence of the same testing doses of acetylcholine as in the experimental series.

A trace of contractions of the leech muscle under the influence of various concentrations of ACh before (a) and after incubation with normal rabbit serum (b) and with serum containing AAChR antibodies (c), is illustrated in Fig. 1. It will be evident that these antibodies blocked the ACh receptors of the smooth muscles of the leech and prevented ACh from acting on them.

Average amplitudes of contraction of the leech dorsal muscle under the influence of various ACh concentrations, before and after incubation of the preparation with AAChR antibodies, are shown in Fig. 2.

The results thus show that AAChR antibodies obtained by immunizing a rabbit with antigenic material from motor-denervated skeletal muscles of the hind limbs of BALB/*c* mice, had a marked atropinelike action on the dorsal muscle of the leech, an object highly sensitive to ACh. Our data also confirm the familiar view that ACh receptors do not possess species-specificity [5-7]. In fact, antibodies against ACh receptors of motor-denervated muscles of BALB/*c* mice blocked ACh receptors of motor-denervated muscles of an animal as far removed as regards its species from the mouse as the leech. By way of preliminary conclusion it can be stated that this action of AAChR antibodies can also be observed on other such well-known laboratory objects as the heart and lungs of the frog.

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ROLE OF ADHESIVE CELLS IN THE MECHANISM OF NATURAL CYTOTOXICITY REACTIONS

A. M. Sorokin, S. B. Cheknev, and L. V. Koval'chuk

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A definite role in the regulation of natural killer (NK) cell activity is played by other lymphocyte populations and also by monocytes, macrophages, neutrophils, and eosinophils [15]. Some of these cells participate directly in natural cytotoxicity (NCT) reactions, whereas others mediate regulatory effects on NK cells, without exhibiting any natural killer properties of their own [9].

Interferons Division, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Department of Immunology, N. I. Pirogov Second Moscow Medical Institute, Ministry of Health of the RSFSR, Moscow.

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