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ACTION OF ANTICHOLINERGIC RECEPTOR ANTIBODIES ON THE FROG HEART

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UDC 612.17.014.46:615.217.34].019:597.8

Key words: anticholinergic receptor antibodies; heart; cholinergic receptors; atropine-like effect.

It has been shown that anticholinergic receptor (ACR) antibodies, obtained by immunization of rabbits with cholinergic receptors isolated from motor-denervated skeletal muscles of Balb/c mice, were able to block the cholinergic receptors of mouse splenic lymphocytes by preventing the action of cholinergic drugs on them [1, 2]. This blockade indirectly modified the functions of the immune receptors [1]. It was also found by the direct immunofluorescence method that specific action by ACR antibodies against cholinergic receptors of B lymphocytes led to the virtually total suppression of interaction of the labeled antiglobulin serum with antigen-binding receptors of B lymphocytes, which confirmed previous data relating to changes in the functional state of the immune receptors of lymphocytes during blockade of mediator receptors [2]. Blocking of cholinergic receptors by ACR antibodies also was found in our experiments on the dorsal muscle of *Hirudo medicinalis* [3].

In the investigation described below, the action of ACR-antibodies was studied on the frog heart — an organ with a high density of cholinergic receptors (mainly of the M type) [5].

EXPERIMENTAL METHOD

Experiments were carried out on 85 autumn—winter frogs of the species *Rana temporaria*, male and female, weighing 60-80 g. The test object was the isolated heart, which was removed and cannulated in the usual way. Ringer's solution for cold-blooded animals was used. Cardiac contractions were recorded under isometric conditions on the revolving drum of an electric kymograph. Experiments on the surviving organ were carried out within a period of 2.5-3 h at room temperature. To denervate the hind limb muscles the sciatic nerve of 350 adult frogs and 500 Balb/c mice was divided. The number of cholinergic receptors in denervated muscles is much greater than in nondenervated muscles [4, 9, 10, 12]. Protein of acetylcholine receptors in its membrane-bound form was isolated and purified by the method in [11]. Chinchilla rabbits were immunized with the preparation thus obtained. The antibody titer at which the sera were used in the experiments in the complement fixation test was 1:1280. The following ACR-antibodies were used: antibodies obtained by immunization of rabbits with cholinergic receptor protein isolated from the motor-denervated muscles of the frogs' hind limbs, and also (for the control) form nondenervated frog hind limbs; antibodies against cholinergic receptors of motor-denervated hind limb muscles of Balb/c mice. Serum of unimmunized intact rabbits also was used as the control. The sera were used in dilutions of 1:10, 1:5, and 1:2, and also undiluted. The experimentally selected incubation time of the test

Central Research Laboratory for Synthesis and Testing of New Drugs, N. I. Pirogov Second Moscow Medical Institute. Institute of Immunology, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 170-174, August, 1991. Original article submitted February 6, 1991.

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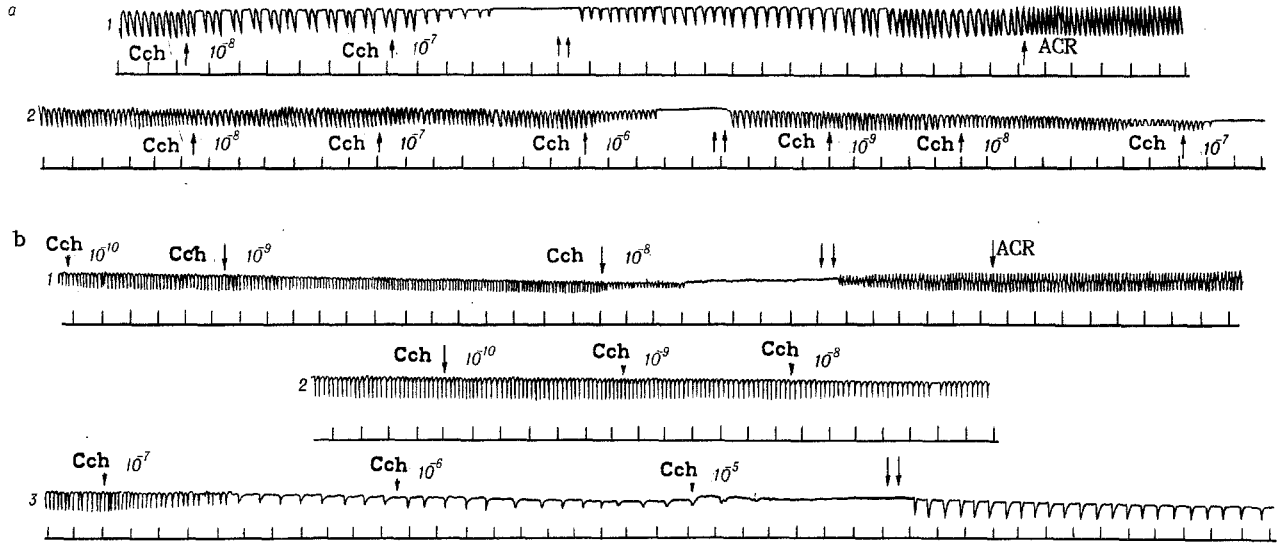

Fig. 1. Effect of ACR antibodies on the frog heart. Cardiac contractions tested against Cch. Heart incubated with antibodies against cholinergic receptor protein isolated from denervated frog muscles. Titer of antibodies in CFT 1:1280. Dilution of ACR-serum 1:5. Incubation time 25 min (a) and 20 min (b). a: 1) Action of Cch before incubation of heart with ACR-antibodies; 2) action of Cch after incubation, followed by repeated washing and repeated action of Cch; b: 1) action of Cch before incubation of heart with ACR-antibodies; 2, 3) after incubation followed by rinsing of the heart. Time marker 10 sec. Here and in Fig. 2, arrow indicates time of administration of drugs, two arrows — time of rinsing heart with Ringer's solution for cold-blooded animals (explanation in text).

object with the ACR-antibodies was 20-25 min. Incubation was carried out at room temperature. Ampul-packed acetylcholine-chloride (Ach), carbachol (Cch) in dilutions of 10^{-14}-10^{-3}, and atropine sulfate in dilutions of 10^{-11}-10^{-3} were used. The preparations were diluted immediately before use.

EXPERIMENTAL RESULTS

In these experiments, a negative inotropic and chronotropic effect on the frog heart was obtained by the action of Ach or Cch in dilutions of 10^{-13}-10^{-12}. Total cardiac arrest occurred when these preparations were used in dilutions of 10^{-10}-10^{-8} or lower. In this case, the phenomenon of "escape" of the heart from the inhibitory influence of cholinergic drugs (the escape phenomenon) was not observed. The time of observation was 4-5 min. Cardiac contractions were restored after the heart had been rinsed with Ringer's solution for cold-blooded animals.

Atropine in a dilution of 10^{-11} considerably weakened the inhibitory effect of Ach or Cch. To cause cardiac arrest after atropinization, the concentration of the cholinergic preparations had to be increased by 4-5 orders of magnitude. The effect of withdrawal of the inhibitory action of Ach or Cch on the frog heart under the influence of atropine was potentiated when it was used in higher concentrations. The action of atropine was manifested virtually immediately after its administration and was easily abolished by rinsing the preparation with Ringer's solution. The effect of ACR-antibodies on the frog heart was assessed in relation to the following parameters.

1. The inhibitory effect of Ach or Cch on activity of the frog heart was unchanged after its treatment with ACR-antibodies. The amplitude of the cardiac contractions was virtually identical before and after incubation of the heart with ACR-antibodies.

2. ACR-antibodies, acting on the heart, led to the appearance of an escape phenomenon. This phenomenon is known to be exhibited as spontaneous and relatively rapid release of the heart from inhibitory cholinergic influences. During the action of ACR-antibodies this phenomenon occurred particularly easily, and in response to concentrations of Ach or Cch at which it did not occur before treatment of the heart with ACR-antibodies.

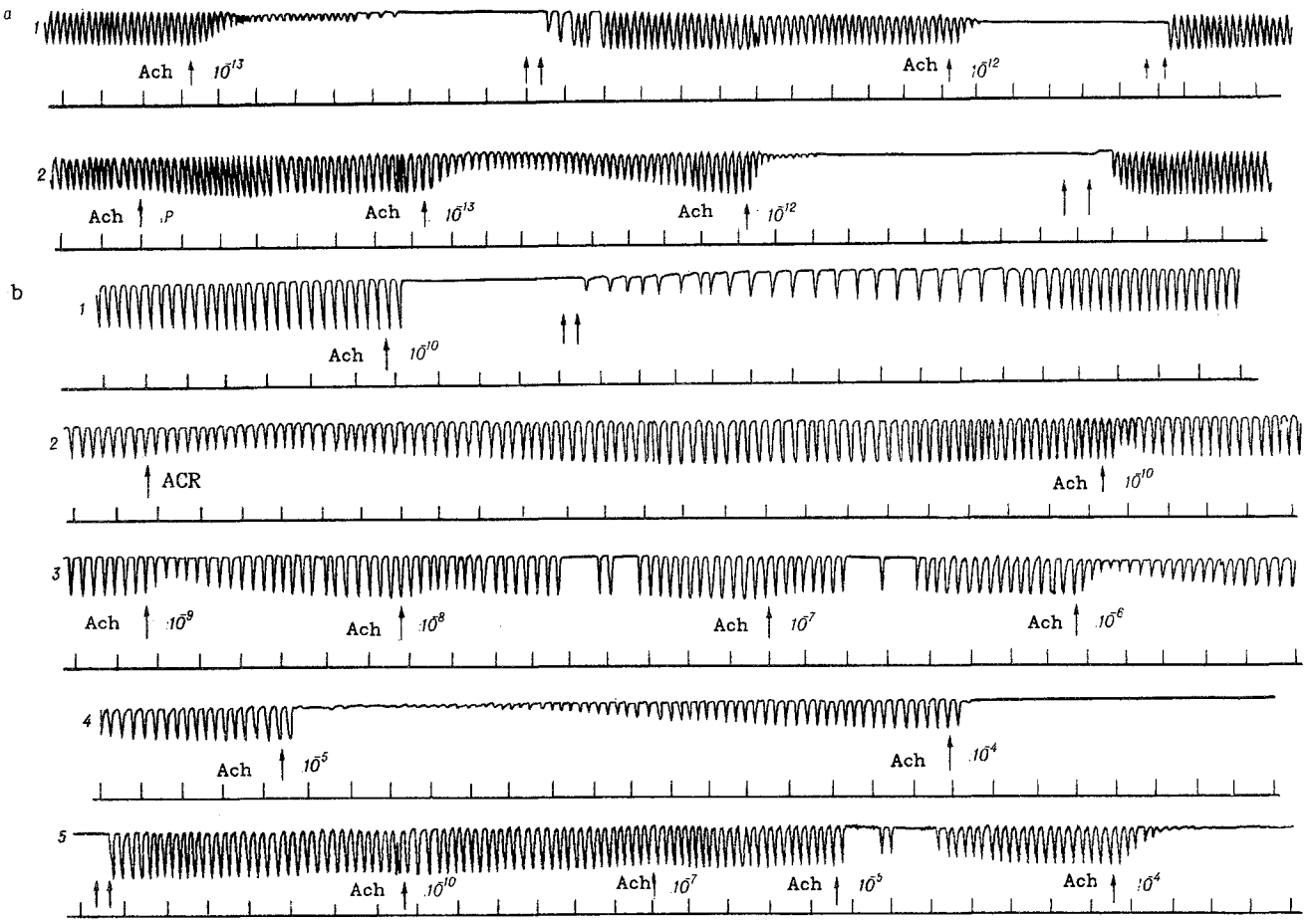

Fig. 2. Action of ACR-antibodies on frog heart. Cardiac contractions tested against Ach. a) Incubation of heart with ACR-antibodies against cholinergic receptors of motor-denervated frog muscles; b) incubation of heart with ACR-antibodies against cholinergic receptors of motor-denervated muscles of Balb/c mice. Incubation time 25 min. Titer of antibodies in CFT 1:1280. Dilution of sera 1:5. Explanation in text.

3. ACR-antibodies caused a sufficiently well marked weakening of the inhibitory effect of Ach or Cch on cardiac activity. Under these circumstances the amplitude of the cardiac contractions was reduced under the influence of the same concentrations of the cholinergic preparations by a much lesser degree than before treatment of the heart with ACR-antibodies, and to produce complete cardiac arrest the concentration of cholinergic preparations had to be an order of magnitude higher than before incubation of the heart with ACR-antibodies.

4. In cases of a well-marked action of ACR-antibodies it was even more difficult to induce the same degree of reduction of amplitude of cardiac contractions by Ach or Cch than in the previous cases. Arrest of cardiac activity could be obtained, however, by using Ach or Cch in concentrations 2 or 3 orders of magnitude higher than before treatment of the heart with ACR-antibodies.

It must be pointed out that serum of normal unimmunized rabbits in our experiments never gave rise to the above effects. Treatment of the frog heart with normal rabbit serum, with the same incubation time and in the same dilutions as in the experimental series, did not lead to any change in the inhibitory effect of the cholinergic preparations on cardiac activity.

The action of ACR-antibodies on the frog heart is illustrated in Fig. 1. Antibodies obtained by immunization of rabbits with cholinergic receptors isolated from motor-denervated frog hind limb muscles, which are enriched with these receptors, were tested. It can be seen (Fig. 1a) that cardiac arrest could be induced before incubation of the heart with ACR-antibodies by the action of Cch in a dilution of 10^{-7}, but after incubation for 25 min, in response to the action of

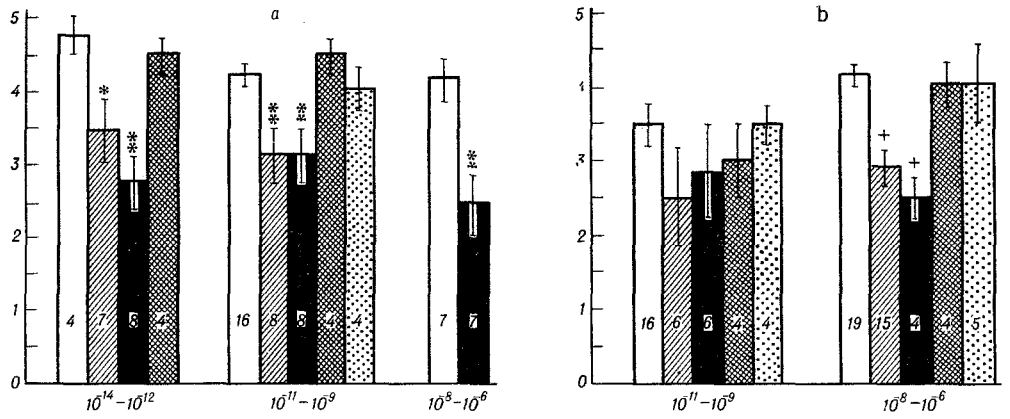

Fig. 3. Effect of Ach and Cch on amplitude of contractions of frog heart before and after treatment with ACR-antibodies. Abscissa, dilution of Ach and Cch; ordinate, amplitude of cardiac contractions (in points, explanation in text). Columns represent mean values (M ± m) of amplitude of cardiac contractions under the influence of Ach (a) and Cch (b). Unshaded columns — before incubation of heart with ACR-antibodies; obliquely shaded columns — after incubation of heart with antibodies against cholinergic receptors of motor-denervated frog muscles; black columns — after incubation of heart with antibodies against cholinergic receptors of motor-denervated muscles of Balb/c mice; cross-hatched columns — after incubation of heart with serum of rabbits immunized with cholinergic receptor protein isolated from nondenervated frog muscles; dotted columns — after incubation of heart with blood serum of intact nonimmunized rabbits. Incubation time of test object with ACR-antibodies 20-25 min. Dilution of sera 1:5. *$p < 0.05$; **$p < 0.01$; $^{+}p < 0.001$.

Cch in a dilution of 10^{-6}. After the heart had been rinsed with Ringer's solution the effect of the ACR-antibodies was abolished with difficulty.

Arrest of the frog heart after incubation for 20 min with ACR-antibodies could be induced only by the action of Cch in a dilution of 10^{-5} (Fig. 1b, 3), whereas it could be arrested before treatment with ACR-antibodies (Fig. 1b, 1) by the action of Cch in a dilution 3 orders of magnitude higher, namely 10^{-8}.

The effect of ACR-antibodies on activity of the frog heart, tested against Ach, is illustrated in Fig. 2. In the first case (Fig. 2a) antibodies against cholinergic receptors of motor-denervated frog muscles were used, in the second case (Fig. 2b) antibodies against cholinergic receptors of motor-denervated muscles of Balb/c mice.

Cardiac arrest before treatment with ACR-antibodies (Fig. 2a, 1) appeared under the influence of Ach in a dilution of 10^{-13}. After incubation for 25 min with ACR-antibodies (Fig. 2a, 2), on the addition of Ach 10^{-13} the heart "escaped" from its inhibitory influence, and its activity was restored spontaneously. Complete cardiac arrest occurred in response to Ach in a dilution of 10^{-12}.

Arrest of the heart before its treatment with antibodies against cholinergic receptors of denervated mouse muscles occurred in response to Ach in a dilution of 10^{-10} (Fig. 2b, 1). Incubation of the heart with ACR-antibodies for 25 min led to considerable weakening of the inhibitory effect of Ach on cardiac activity. For instance, under the influence of Ach in dilutions of 10^{-10}-10^{-6} (Fig. 2b, 3) rapid release of the heart from the inhibitory influence of the drug (the escape phenomenon) took place. Even with Ach in a concentration of 10^{-5}, the heart was still in a state of release from its influence (Fig. 2b, 4). Complete cardiac arrest was observed in response to Ach in a dilution of 10^{-4} (Fig. 2b, 4). Thus to produce cardiac arrest the concentration of the drug had to be increased by 6 orders of magnitude. After the heart had been rinsed with Ringer's solution for cold-blooded animals, the atropine-like effect of the ACR-antibodies could not be abolished (Fig. 2b, 5).

The result of statistical analysis of the data for testing cardiac activity against Ach (a) and Cch (b) before and after treatment of the heart with ACR-antibodies is illustrated in Fig. 3.

Changes in the amplitude of the cardiac contractions are expressed in points. A 5-point scale of assessment was used: 1 point) the amplitude remained unchanged; 2 points) the escape phenomenon appeared — amplitude was reduced, sometimes to 0, and this was followed by spontaneous recovery; 3 points) reduction of amplitude to 50% of the initial level; 5 points) cardiac arrest without spontaneous recovery. The score in points is inversely proportional to the amplitude of cardiac contractions. All the results were compared with the average value of the amplitudes under the influence of cholinergic preparations before incubation of the heart with ACR-antibodies (Fig. 3, unshaded columns).

It will be clear from Fig. 3a that when cardiac activity was tested against Ach in dilution of 10^{-14}-10^{-12} the inhibitory effect of the drug after incubation of the heart with antibodies against cholinergic receptors of denervated frog muscles (Fig. 3, obliquely shaded columns) was significantly lower than in the control tests ($p < 0.05$). The same effect was observed after incubation of the heart with antibodies against cholinergic receptors of motor-denervated muscles of Balb/c mice (Fig. 3, black column). When inhibitory effects of Ach on the frog heart were compared before and after treatment with the serum of rabbits immunized with cholinergic receptors isolated from nondenervated frog muscles (Fig. 3, cross-hatched columns) no statistically significant difference could be observed (Fig. 3a).

When Ach was used in dilutions of 10^{-11}-10^{-9}, and also 10^{-8}-10^{-6}, the same rule was found: incubation of the heart with different types of ACR-antibodies significantly ($p < 0.01$) weakened the inhibitory effect of Ach on cardiac activity.

Testing cardiac contractions against Cch in dilutions of 10^{-11}-10^{-9} and 10^{-8}-10^{-6} is illustrated in Fig. 3b. Just as with Ach, treatment of the heart with ACR-antibodies led to weakening of the inhibitory action of Cch. Moreover, a significant reduction ($p < 0.001$) was observed, in contrast to experiments with Ach, when high concentrations of Cch were used (10^{-8}-10^{-6}). With low concentrations (10^{-11}-10^{-9}) the differences were not significant.

The reason for this may evidently be that Ach, being a natural neurotransmitter, can induce not only more clearly defined, but also more easily abolished effects than an artificial cholinergic drug such as Cch.

In the control series of experiments with blood serum of normal, nonimmunized rabbits (Fig. 3, dotted columns), and also with the blood serum of rabbits immunized with cholinergic receptors isolated from nondenervated frog muscles (Fig. 3, cross-hatched columns), we did not find the effect observed under the influence of ACR-antibodies.

Our investigations showed that ACR-antibodies can exert a specific action on the cholinergic receptors of the frog heart. This action prevented the effect of Ach and Cch on cholinergic receptors, or greatly weakened it. The inhibitory effect of cholinergic drugs on the heart under these circumstances was significantly reduced. Unlike the action of atropine, this effect persisted longer and was abolished with difficulty (sometimes not at all) after rinsing the heart with Ringer's solution. However, the reproducibility of abolition of the inhibitory effect under the influence of ACR-antibodies was lower than under the influence of atropine. ACR-antibodies thus possess an atropine-like effect, which was manifested as their ability to abolish, to a greater or lesser degree, the inhibitory influence of Ach and Cch on the frog heart. This evidently took place through competition between ACR-antibodies and cholinergic receptors of denervated muscles both of the frog and the mouse — an animal of a species far removed from the frog — possess this atropine-like effect. This confirms the current opinion that cholinergic receptor and ACR-antibodies are relatively species-nonspecific [6-8].

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