# CYTOTOXIC ACTION OF ANTIBODIES ON MYOCARDIAL ELECTRICAL ACTIVITY IN THE ABSENCE OF COMPLEMENT. THE PROTECTIVE EFFECT OF HEPARIN

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Inactivation of the complement in a rabbit antiserum against guinea pig myocardium by the action of heat does not abolish the cytotoxic action of the antibodies on myocardial electrical activity. Experiments on the isolated heart (perfused via the coronary system, ECG recorded by macroelectrodes) revealed sinus or nodal tachycardia, atrioventricular block, atrial arrest, etc., 4-6 min after injection of the antiserum into the perfusion fluid. In a preparation of the spontaneously contracting auricle of the atrium (external perfusion, potentials recorded by intracellular microelectrodes), an initial increase in spike frequency followed by a decrease, disturbance of the rhythm, and cessation of spontaneous activity were observed during the action of antiserum in a dilution of 1:5-1:20. All these effects of the antibodies were partly or completely abolished when heparin (10-20 units/ml) was injected into the perfusion fluid containing the antiserum. It was concluded that heparin has not only anticomplementary action, but also a direct anticytotoxic action.

Modern views on the cytotoxic action of antibodies and the leading role of complement in these reactions [3-6, 8] are based mainly on the results of morphological investigations (electron microscopy, phase-contrast analysis, histochemistry, etc.). However, investigations of this type can reveal only the comparatively gross and advanced disturbances of the structure of cell membranes. The initial molecular changes in this structure, which determine the functional properties of membranes (their ionic permeability, potentials, and so on) are far beyond the limits of resolving power of these methods.

In the investigation described below, with the object of studying the action of specific antiserum on the guinea pig heart, electrophysiological indices recorded by extracellular and intracellular electrodes were accordingly chosen as indicators of the cytotoxic effect.

## EXPERIMENTAL METHOD

Experiments with extracellular recording of the potentials (ECG) were carried out on the whole guinea pig heart perfused through the coronary system with Tyrode solution (composition in mM: NaCl 136.7, KCl 2.69, $CaCl_2$ 1.8, $NaHCO_3$ 1.9, $NaH_2PO_4$ 0.39, glucose 11.1), aerated by a mixture of 95% $O_2$ and 5% $CO_2$, and heated to 37°C.

The heart was placed in a transparent plastic chamber with silver recording electrodes mounted in its walls and connected to a type ELKAR-4 electrocardiograph. In some of the experiments the recording electrodes were secured to the surface of the atria and ventricles by means of MK-6 glue.

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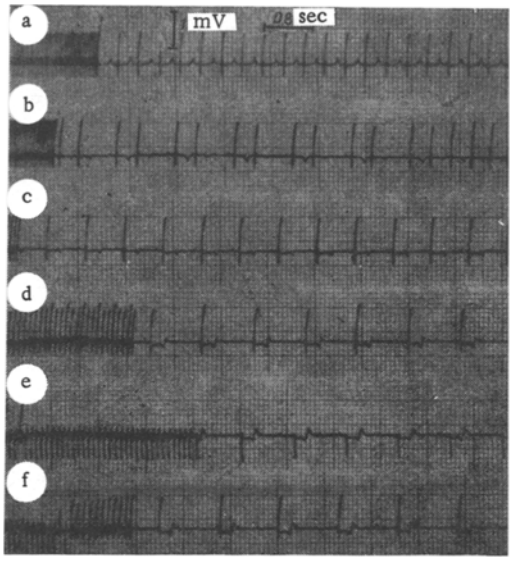


Fig. 1. Changes in ECG of guinea pig heart under the influence of antiserum and restorative effect of heparin. Complement inactivated by heat: a) initial background, heart perfused via coronary system with Tyrode solution; b) 5 min after injection of antiserum into coronary circulation; against the background of sinus tachycardia, periodic (3 : 2) atrioventricular (AV) block; c) 6 min after injection, 2 : 1 AV block; d) 12 min after injection, complete AV block, every 3rd ventricular complex is a nodal extrasystole; e) 13 min after injection, appearance of heterotopic pacemakers in upper part of bundle of His; f) 14 min after injection of antiserum and 1 min after addition of heparin (10 units/ml) to solution, restoration of pacemaker activity of AV node. ECG recorded by electrodes immersed in solution bathing the heart.

The experiments with intracellular recording of the potentials were carried out on a preparation of the isolated auricle of the right atrium, contracting spontaneously. The preparation was placed in a glass chamber through which Tyrode solution was passed. The potentials were recorded by glass microelectrodes filled with 2.5 M KCl solution, from which they were fed to a cathode follower (Nihon Kohden, Japan) and 2-channel dc amplifier ("Disa" Universal indicator). The anticardiac serum was prepared by immunizing rabbits with 20% saline extract of guinea pig heart tissue, previously freed from traces of blood. The resulting sera reacted in the complement fixation test in dilutions of 1 : 80-1 : 320, and in the ring-precipitation test with homologous antigen diluted to a protein concentration of 15-30 $\mu$g. In both series of experiments the antisera were diluted 1 : 5-1 : 20 with Tyrode solution. In the control experiments normal serum of unimmunized rabbits was used.

To inactivate the complement the test serum was heated at 56°C for 30 min. Heparin was used in concentrations of 5-20 units/ml.

## EXPERIMENTAL RESULTS AND DISCUSSION

In all 22 experiments on the whole heart, injection of anticardiac serum into the perfusion fluid caused marked disturbances of electrical activity of the heart. The ECG changes in the control experiments with normal serum were slight in degree and transient in duration.

A cytotoxic effect was found as early as 4-6 min after the beginning of perfusion: sinus or nodal tachycardia appeared, harmony between the working of the ventricles and atria was lost, partial or complete atrioventricular block developed, and heterotopic foci appeared in conjunction with atrial arrest (Fig. 1a-e). The less diluted the antiserum given, the more marked these changes were.

Heparin had a definite protective action. If the heparin was added to the perfusion solution when changes in the ECG were already established (Fig. 1f), the changes were reversed: a regular rhythm of excitation appeared, atrioventricular conduction was partly or completely restored, and the amplitude of the ECG waves increased (8 experiments). If, however, the antisera were added to the perfusion solution containing heparin (10 units/ml), the cytotoxic effect was either absent (in 4 of 8 experiments) or weak and transient (4 experiments).

The preparation of the atrial auricle was perfused initially with Tyrode solution of normal composition (Fig. 2a). The amplitudes of the resting potential and action potential and the maximal gradient of the ascending phase were 52.2 ± 8.4 mV, 64.9 ± 7.8 mV, and 92.2 ± 21.9 V/sec, respectively (45 fibers were tested). The firing rate varied from 69 to 160/min.

Replacement of the Tyrode perfusion solution by solution containing antiserum in most experiments caused initially a marked increase in the frequency of the spontaneous activity (Fig. 2b), followed by slowing of the activity and arrhythmia (Fig. 2c), leading ultimately to complete abolition of spontaneous activity (Fig. 2d). The firing rate in the initial stage of action of the antiserum was usually increased by 2-2.5 times, to reach 300-350/min in certain cases. The duration of the action potentials was sharply reduced under these conditions. In some experiments no increase in frequency occurred, but there was a decrease

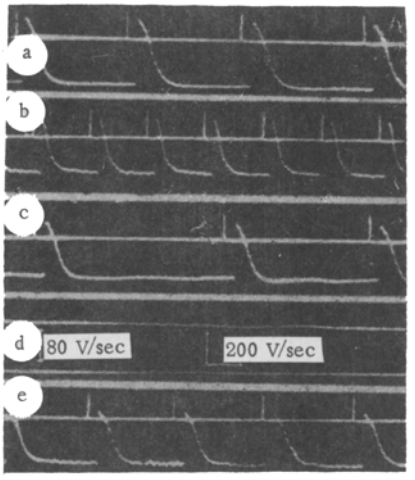


Fig. 2

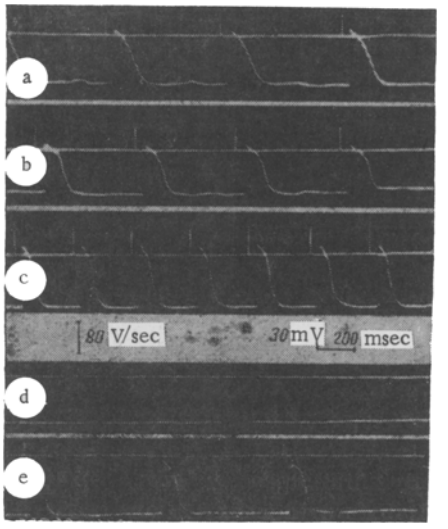


Fig. 3

Fig. 2. Changes in spontaneous activity of single atrial fibers (right auricle) under the influence of antiserum. a) Initial background in Tyrode solution: spreading action potential from pacemaker (bottom line), maximal gradient of ascending phase of spike (top line; for convenience of reading the record is shifted to the left), and resting potentials (distance between top and bottom lines in interval between spikes); b) stage of increased frequency; c) stage of decreased frequency and arrhythmia; d) cessation of spontaneous activity; e) preparation rinsed in Tyrode solution. Experiment on July 9, 1970.

Fig. 3. Protective effect of heparin: a) initial background (the same as in Fig. 2a); b) combined administration of antiserum (1 :15) and heparin (10 units/ml); c) above solution replaced by antiserum solution (1:15) without heparin; experiment on July 14, 1970; d) cessation of spontaneous activity under the influence of antiserum (1 :10); e) restoration of spontaneous activity on addition of heparin (10 units/ml) to the solution. Experiments on July 13, 1970.

from the very beginning, to be followed by complete disappearance of the action potentials. It is interesting that during all these changes in electrical activity the resting potential of the myocardial cells remained virtually constant. For instance, in the period of cessation of spontaneous activity the resting potential of the atrial fibers was $53 \pm 6.1$ mV (10 fibers). In most cases these effects were reversible: rinsing the preparation with Tyrode solution led to partial or complete recovery of electrical activity (Fig. 2e).

Heparin in concentrations of 5-10 units/ml itself caused no significant changes in the electrical activity of the atrial fibers but had a definite protective action when used in conjunction with antiserum. This was manifested either as prevention of the cytotoxic effect if the serum was added to perfusion solution already containing heparin (Fig. 3a, b) or as abolition of the already developed cytotoxic reaction if the heparin was added to the solution after the serum had begun to act (Fig. 3d, e). With this concentration of heparin, the degree of its protective action was directly dependent on the degree of dilution of the antiserum. In the experiments in which rinsing the preparation in Tyrode solution did not restore electrical activity, it was restored by the addition of heparin.

Since in these experiments the complement contained in the antiserum had been inactivated by preliminary heating, the observed changes in electrical activity can be interpreted as the result of the direct action of antibodies on the excitable cell membranes.

The similarity between the changes in electrical activity of the myocardial cells in the initial stage of the cytotoxic reaction (the phase of tachycardia) and those previously found during local anaphylaxis of

these cells [1] was noted. The reaction between antibodies and antigen on the surface of the membrane evidently had a marked effect on the state of its sodium and potassium canals. Evidence of this was given by changes in the spike frequency, the cessation of spontaneous activity, the shortening of the action potentials, and the blocking of atrioventricular transmission.

The protective action of heparin deserves special attention. Heparin is known to block complement [7] and thus to protect cells against its destructive action. In the present experiments, however, the complement has been inactivated by heat. It thus follows that under these particular conditions heparin has a direct inhibitory effect on the development of the cytotoxic reaction. The mechanism of this protective action of heparin is not yet clear. The simplest explanation would be that heparin prevents the reaction between the antibodies and cell antigens. However, information in the literature on this problem is conflicting: some workers found no effect of heparin on adsorption of antibodies on the cell surface [11,12], while others, on the other hand, report that this adsorption is appreciably weakened in the presence of heparin [9, 10]. The possibility likewise cannot be ruled out that heparin, with its high negative charge [2, 7], can somehow or other counteract the molecular changes which develop in the membrane during antigen–antibody interaction. Be that as it may, it must be accepted that besides its anticomplementary action, heparin also possesses a direct antitoxic action.

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