INTRACELLULAR INVESTIGATION OF AN ANAPHYLACTIC REACTION OF GUINEA PIG ATRIAL CELLS

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Changes in the electrical activity and membrane resistance of various types of atrial cell of guinea pigs were investigated in vitro during an anaphylactic reaction.

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The work of A. D. Ado and his collaborators led to formulation of the hypothesis that allergic changes take place in the smooth-muscle cells themselves during allergic reactions of smooth-muscle organs [1-3]. It was therefore interesting to study the electrical properties of the membrane of muscle cells during an allergic reaction. Myocardial cells provide a suitable model for such an investigation, for there is reason to suppose that the anaphylactic reaction of myocardial and smooth-muscle cells obeys, in principle, the same laws and that myocardial cells, like smooth-muscle cells, can be a direct target for action of an antigen or antigen–antibody complex [9, 13]. Meanwhile the study of an anaphylactic reaction on the heart also enables a separate analysis to be made of the pacemaker function and of activation of the contractile elements, which are divided among different cells in the heart, unlike these functions in smooth-muscles, which are performed by the same cells, although possibly by different areas of the cell membrane [12].

In this paper results are described showing changes in the rhythmic activity and membrane resistance of two types of atrial fibers–pacemaker and contractile–of guinea pigs during an anaphylactic reaction.

EXPERIMENTAL METHOD

Experiments were carried out on 31 male guinea pigs weighing 250-300 g. The animals were sensitized as described earlier [2]. The experiments began 4 weeks after the last sensitizing injection of antigen, on the isolated atria [7], which were placed in a special chamber for isolated organs, with a capacity of 6 ml. Constant perfusion with Kreb's solution [2], saturated with oxygen at the rate of 2 ml/min, was maintained during the experiments. The temperature of the fluid was 37°. The glass microelectrodes used in the work were filled with 3M KCl and their impedance was between 10 and 50 MΩ. A "Disa Electronic" electrometer with high-input impedance and compensation of input capacitance was used. To determine the cell resistance a bridge method was used, enabling a polarizing current to be applied to the cell and potentials from it to be picked up by the same microelectrode [4, 5, 8]. The principle underlying this method is that the amplitude of the action potential changes when a weak polarizing current is applied to the cell by an amount practically equal to the change in resting potential, for the resistance of the cell membrane is considerably reduced at the peak of the action potential, amounting in the case of the giant axon, the muscle fibers of the frog, and single cardiac fibers, for example, to less than 5% of the membrane resistance at rest [6, 10, 14]. Hence, the amount by which the amplitude of the action potential changes is essentially equal to the voltage drop at the membrane resistance caused by application of the polarizing current to the membrane.

Altogether about 250 cells of the right atrium were studied.

EXPERIMENTAL RESULTS AND DISCUSSION

Types of electrical activity of the various types of atrial cells have been studied reasonably well [11]. The electrical activity of cells of pacemaker type consists of slow depolarization of the membrane in the

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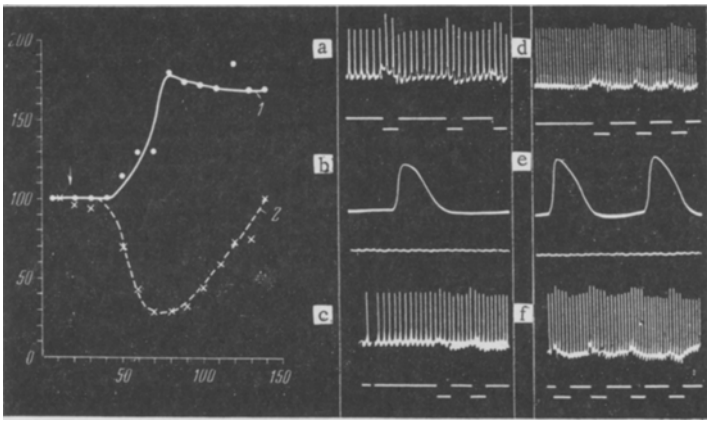

Fig. 1. Anaphylactic reaction of pacemaker of right atrium of a guinea pig. The graph shows changes in frequency of action potentials (1) and membrane resistance (2) as percentages of initial values. Ordinate—percentages, abscissa—time of recording (in sec). The arrow denotes the time of addition of antigen. Traces a and b correspond to the 10th, c to the 60th, d and e to the 90th, and f to the 120th second of recording. Time marker for a, c, d, and f 1 sec, for b and e 40 sec. Calibration signal 10 mV. Upward deflection of beam corresponds to depolarization. Strength of pulses of hyperpolarizing current $4 \cdot 10^{-9}$ A.

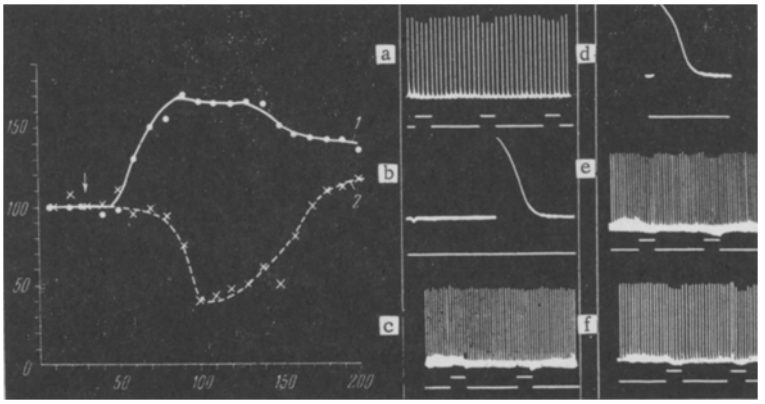

Fig. 2. Anaphylactic reaction of contractile fiber of the right atrium of a guinea pig. Traces a and b correspond to 10th, c and d to 100th, e to 120th, and f to 160th second of recording. Strength of pulses of depolarizing current $4 \cdot 10^{-9}$ A. Remainder of legend as in Fig. 1.

interspike period, until the threshold is reached, when an action potential is produced with a fairly slow buildup period (Fig. 1, b). The atrial contractile fibers are characterized by a stable membrane potential in the interspike period, by a fast buildup of the action potential, and by a relatively prolonged phase of repolarization (Fig. 2, b).

Changes in the action potentials and membrane resistance of the pacemaker during development of an anaphylactic reaction of the atrium are illustrated in Fig. 1. The initial pattern consists of action potentials with a frequency of about 150/min, characteristic of the true pacemaker. The duration of slow depolarization in the period preceding the action potential is 286 msec, and its rate 0.028 V/sec. The duration of the buildup period is 27 msec, and of the repolarization phase 124 msec. On the graph the change in

frequency of the spontaneous action potentials is plotted as a percentage of the initial value, taken as 100. The frequency of the action potentials increased considerably 30 sec after addition of the specific antigen (ovalbumin, 100 μg/ml), reaching a maximum by 60-70 sec. Against the background of the maximal increase of frequency, no appreciable change took place in the duration of the buildup and repolarization periods of the action potential. Meanwhile, the slow depolarization in the period preceding the action potential was speeded up considerably, its rate reaching 0.99 V/sec. The threshold level of the potential also fell. Changes in the relative values of the membrane resistance of the test cell are also illustrated in Fig. 1. The increase in amplitude of the spontaneous action potentials during application of the hyperpolarizing current ($4 \cdot 10^{-9}$ A) to the cell before addition of antigen was taken as 100. Subsequent changes in the increase of amplitude of the action potentials during application of a hyperpolarizing current of the same strength are expressed as percentages of the initial value. It is clear from Fig. 1 that almost simultaneously with the development of an anaphylactic reaction of the atrium, as judged from the increase in frequency of the spontaneous action potentials [7], the membrane resistance fell significantly. This decrease reached its greatest value after 50-60 sec, after which the membrane resistance gradually returned to its initial value. The frequency of the action potentials remained higher than initially.

Changes in the same indices of electrical activity of the membrane of an atrial contractile fiber during an anaphylactic reaction are illustrated in Fig. 2. The frequency of the spontaneous action potentials was increased 30 sec after addition of the antigen, reaching a maximum (up to 160-170%) by 70 sec. It will be seen in Fig. 2 that the changes in spontaneous action potentials are characterized by slowing of the buildup period, lengthening of the repolarization phase, and some decrease in amplitude. Development of the anaphylactic reaction of the contractile atrial fiber likewise was accompanied by a marked decrease in resistance of the cell membrane after addition of antigen. The relative value of the membrane resistance of the cell was restored before the initial frequency of the spontaneous action potentials.

The results of more recent investigations [9, 13] have shown that the myocardial cells of sensitized guinea pigs may be the direct target for action of a specific antigen. These findings have been confirmed morphologically: fixation of the antigen on the surface of sensitized myocardial cells has been demonstrated [9]. It may therefore be concluded that the mechanisms of development of the anaphylactic reaction of smooth-muscle and myocardial cells are analogous in principle [3].

The experimental results demonstrate that the character of electrical activity of smooth-muscle and atrial fibers during an anaphylactic reaction corresponds to the state of excitation of these units during excitation by other factors. For atrial fibers of pacemaker type these changes during the anaphylactic reaction consist of an increase in the frequency of the spontaneous action potentials, due to two mechanisms simultaneously: acceleration of the slow depolarization in the period preceding the action potential and lowering of the threshold potential level. Changes in the electrical activity of the atrial contractile fibers consist of an increase in frequency of the spontaneous action potentials. The amplitude of the action potentials is slightly reduced in these circumstances, the buildup period is lengthened, and the repolarization phase is increased, in agreement with published data [7, 13]. As a continuation of the present investigation, the relative values of the membrane resistance of the types of atrial cells investigated were determined during an anaphylactic reaction. At the moment of development of the anaphylactic reaction of the atrium a transient decrease in resistance of the membrane developed both in fibers of pacemaker type and in the contractile fibers. In both cases the duration of the decrease in membrane resistance did not coincide with the duration of the period of increase in frequency of the spontaneous action potentials, and the membrane resistance was restored much sooner than the frequency of the action potentials.

The results relating to changes in resistance of the atrial cell membranes may be compared with those obtained by investigating the ionic permeability of smooth-muscle during an anaphylactic reaction [1]. The decrease in resistance of the membrane of the investigated cells is perhaps an expression of increased permeability of the membrane to potassium ions, in turn brought about by the modifying action of the antigen [1].

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