ANALYSIS OF MECHANISMS OF THE POSITIVE INOTROPIC ACTION OF A PROTEIN ANTIGEN (EGG ALBUMIN) AND HISTAMINE ON THE MYOCARDIUM OF SENSITIZED GUINEA PIGS

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Changes in contractile activity of the myocardium during the local anaphylactic reaction and the action of histamine were investigated in preparations of the left auricle of guinea pigs sensitized beforehand with egg albumin. Histamine ($1 \cdot 10^{-5}$ g/ml) and egg albumin ($2 \cdot 10^{-5}$ g/ml) caused little change in the first (after 3 min of rest) contraction of the preparation, but considerably enhanced the increase in amplitude of contractions in a rhythmic series (positive "Bowditch's ladder"). The higher the frequency of stimulation (between 1 and 5 Hz) of the preparation, the stronger the positive inotropic effect of histamine and egg albumin. Inactivation of fast Na channels by depolarization of the membrane of the myocardial cells in medium with a high potassium concentration (13.7 mM KCl) did not abolish these effects of histamine and the antigen. Conversely these effects were completely suppressed by blocking of the slow Na, Ca channels by compound D-600 ($1 \cdot 10^{-6}$ g/ml).

KEY WORDS: anaphylaxis; myocardium; ion transport; histamine; contraction.

This investigation is a continuation of studies of the ionic mechanisms of the local anaphylactic action of heart muscle commenced previously [1, 3, 4]. On the basis of the study of the effect of an antigen (egg albumin) and histamine on intracellular potentials of the guinea pig auricle it has been suggested [1] that during interaction between antigen and antibody on cell membranes, just as during the direct action of histamine, activation of slow Na, Ca channels takes place in the membrane of the myocardial cells. However, in the investigations cited above insufficient attention was paid to the study of changes in the contractile properties of the myocardium during these procedures.

In the present investigation a special study was made of this problem. The experiments were carried out when the fast Na and slow Na, Ca channels were functioning normally and also when they were blocked separately: The former were inactivated by moderate potassium depolarization of the membrane, the latter by means of compound D-600.

EXPERIMENTAL METHODS

The auricle of a guinea pig previously actively sensitized [7] was placed in a chamber and perfused with oxygenated (96% O_2 + 4% CO_2) Tyrode solution at 33-35°C. One end of the auricle was fixed in the chamber, the other was attached by means of a thread to a 6MKh1S mechanotron. The frequency of contraction of the preparation was assigned by an ÉSU-1 stimulator. The duration of the repetitive series of stimuli in all cases was 15 sec and was followed by a period of rest lasting 3 min; only then was a new series of stimuli with a different frequency applied. Frequencies were used in the following order: 1, 2, 3, 4, and 5 Hz. The action of each solution was tested for 20-60 min, during which period 2 or 3 recordings were obtained of the rhythmic series of contractions which developed to each frequency ("Bowditch's ladder").

The action of egg albumin ($2 \cdot 10^{-5}$ g/ml) and histamine ($1 \cdot 10^{-5}$ g/ml) was studied under the following conditions: in Tyrode solution of the ordinary composition (five experiments), in Tyrode solution containing D-600 in a concentration of 10^{-6} g/ml (four experiments), and in a solution with the KCl concentration increased to 13.7 mM (16 experiments). The pH of all solutions was 7.2-7.4.

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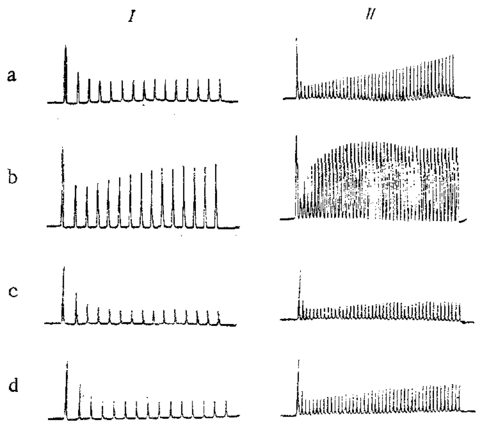

Fig. 1. Effect of egg albumin on contractions of "Bowditch's ladder" of left auricle in a sensitized guinea pig. Frequency of stimulation 1 Hz (I) and 3 Hz (II). a) "Bowditch's ladder" in normal Tyrode solution; b) first addition of antigen in a concentration of $2 \cdot 10^{-5}$ g/ml; c) 15 min after beginning of perfusion with solution containing antigen; d) second addition of antigen in the same dose. Records of one experiment.

EXPERIMENTAL RESULTS

The addition of egg albumin to normal Tyrode solution perfusing the auricle of a sensitized guinea pig had a positive inotropic effect in all experiments; as a rule this effect was more marked during stimulation with a frequency of 3 Hz than with a frequency of 1 Hz. The results of one experiment of this series are illustrated in Fig. 1. Under the influence of the antigen and with stimulation at a frequency of 1 Hz the "ladder" changed from negative to positive (Fig. 1, Ia and b), but at a frequency of 3 Hz there was a sharp increase in amplitude of the contractile responses in the rhythmic series (Fig. 1, IIa and b). The positive inotropic effect in this experiment lasted about 15 min, after which, as the action of egg albumin continued, the contractions fell even below the initial level (Fig. 1, Ic and IIc). After rinsing out of the antigen, its application a second time (Fig. 1, Id and IId) was virtually ineffective, indicating desensitization of the preparation. In the other experiments of this series the duration of the positive inotropic effect varied from 12 to 17 min. An increase in the amplitude of the first contraction took place in 3 of 5 experiments; the increase (12-30%) was smaller than the increase in the last contraction in the series (100-172% at a frequency of 1 Hz and 118-224% at 3 Hz).

In five experiments the action of egg albumin ($2 \cdot 10^{-4}$ or $2 \cdot 10^{-5}$ g/ml) on the atrium of a guinea pig which had not been previously sensitized was tested as a control. In all the experiments of this series the addition of albumin to the solution caused a decrease (by 5-40%) in the amplitude of contraction.

Egg albumin and histamine had a marked positive ionotropic action on the sensitized preparation when administered during potassium depolarization of the membrane also. In these experiments the concentration of potassium ions in the Tyrode solution was increased to 13.7 mM. The shape of the "Bowditch's ladder" of the preparation partly depolarized by a solution with an increased concentration of K^+ differed only a little in principle from that in normal Tyrode solution. Here also the first contraction in the rhythmic series was the strongest, and the time required for the contraction to increase to the maximum, and the actual value of the maximum depended on the frequency of stimulation. This is clearly visible in Fig. 2 if curves 1 of the top series are compared. These curves are envelopes of "Bowditch's ladder" for stimulation of the preparation at frequencies of 1, 2, and 4 Hz (Fig. 2: A1, B1, and C1 respectively). Addition of the antigen and histamine to the solution with an increased K^+ concentration in 14 experiments caused an increase in the amplitude of all contractions ($P < 0.01$). In the presence of an excess of K^+, even more than in Tyrode solutions, the difference between the relative changes in the first and last contractions under the influence of antigen and histamine became perceptible. The first contraction was strengthened only a little, by not more than 8%, whereas the increase in the last contraction reached 150-400%. The positive ionotropic effect of these agents also was manifested as an increase in the steepness of the "ladder" (see Fig. 2: A2, B2, C2). After perfusion of the preparation for 15-20 min with antigen the signs of the inotropic effect gradually disappeared, indicating

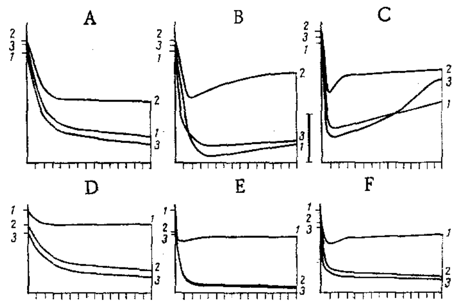

Fig. 2. Changes in shape of "Bowditch's ladder" under the influence of egg albumin and histamine during potassium depolarization of the preparation (A, B, C) and blocking of slow Na, Ca channels by compound D-600 (D, E, F). Top row: envelopes of "Bowditch's ladder" during stimulation at frequencies of 1 (A), 2 (B), and 4 Hz (C); 1) "ladders" in Tyrode solution with KCl concentration of 13.7 mM, 2) immediately after addition of egg albumin ($2 \cdot 10^{-5}$ g/ml) to solution, 3) 22 min after beginning of perfusion with albumin. All results in top row obtained from same experiment. Bottom row: at frequencies of stimulation of 1 (D) and 3 Hz (E, F); 1) "ladders" in Tyrode solution of normal composition, 2) after action of D-600 ($1 \cdot 10^{-6}$ g/ml) for 20 min, 3) 5-7 min after addition of egg albumin (D, E) or histamine (F) to D-600 solution. Bottom row shows results of three experiments: D, E, and F. Marks on abscissa is time scale (1 sec); ordinate gives calibration of muscular contraction, in units of 100 mg.

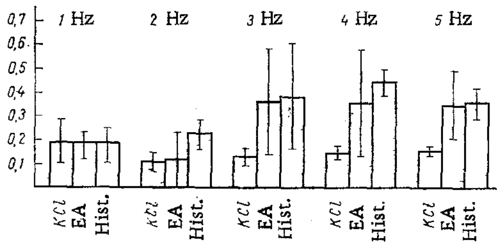

Fig. 3. Changes in ratios of last contraction developing during 15 sec of "Bowditch's ladder" and first contraction during action of KCl, egg albumin (EA), and histamine (Hist) against the background of an increased KCl concentration, depending on frequency of stimulation.

desensitization of the preparation. In this particular experiment (Fig. 2), after perfusion of the preparation with antigen for 22 min its effect completely disappeared at frequencies of stimulation of 1 and 2 Hz, but could still be detected during stimulation with a frequency of 4 Hz (Fig. 2: A3, B3, C3).

For quantitative analysis of the positive inotropic effect of histamine and the antigen, the ratio of the amplitude of the last contraction in the rhythmic series to the first was used (it was stated above that the first contraction showed only a very small change under the influence of these agents). Changes in this ratio under the influence of histamine and the antigen and at different frequencies of stimulation are shown in Fig. 3. Clearly the ability of the two agents to activate rhythmic contraction of the preparation depended on the frequency of stimulation and the greatest effect was obtained at 3, 4, and 5 Hz.

The results described above give the answer to the question why, when clear changes were observed previously in the action potential under the influence of antigen and histamine on a sensitized preparation depolarized by KCl solution, an increase in the contractions was not observed [1, 3]. In the investigations cited the interval between stimuli varied and was always longer than 3 sec. Meanwhile, as the present investigation showed, the positive inotropic action of histamine and of the antigen is found only during fast repetitive stimulation of the preparation. The reason for this is probably the parallel development of positive chronotropic and inotropic effects during the local anaphylactic reaction and during the action of histamine, observed on a spontaneously active preparation of the auricle [2, 8].

Finally, the action of the antigen and histamine on a preparation of myocardium was tested during blocking of the slow Na, Ca channels by compound D-600. In four experiments of this series D-600 was added to normal Tyrode solution. Under the influence of D-600 the positive "Bowditch's ladder" disappeared; the higher the frequency of stimulation of the preparation, the more marked was the decrease in amplitude of the rhythmic contraction (see Fig. 2: D2, E2, F2) [5, 6, 10, 11]. The addition of egg albumin or histamine to the solution containing D-600 caused no positive inotropic effect.

According to existing views the force of contraction developed by any muscle fiber is determined by the concentration of free Ca ions in the myoplasm. In myocardial cells of warm-blooded animals Ca ions enter the myoplasm during each action potential both from the external medium (along Na, Ca channels) and from the lateral cisterns of the sarcoplasmic reticulum. The increase in amplitude of the contractions in the rhythmic series can be explained on the grounds that both portions of Ca^{++} which enter the cell with each action potential gradually accumulate in the reticulum [9]. D-600 weakens the flow of Ca^{++} entering via the Na, Ca channels and thereby reduces the amplitude of the contractions and converts a positive "Bowditch's ladder" to negative [5, 10, 11].

Histamine and the antigen (acting on the sensitized preparation) enhanced the increase in amplitude of contraction in the rhythmic series, and the higher the frequency of stimulation, the greater its effect. It must be emphasized that this effect was also observed during potassium depolarization of the membrane, when the fast Na channels were inactivated. This is, therefore, further evidence in support of the earlier hypothesis [1, 3, 4] that during the local anaphylactic reaction and the action of histamine, slow Na, Ca channels are activated.

The writers postulated previously [5] that, together with electrically excitable Ca channels, constantly open Ca channels also exist in the membrane of the atrial myocardial cell of warm-blooded animals, along which Ca ions continuously pass through the resting membrane into the myoplasm (resting Ca channels). The inward flow of Ca ions along these channels slowly fills the sarcoplasmic reticulum, as a result of which the first contraction after a period of rest in the atrium has a high amplitude. The fact that D-600 has only a weak inhibitory action on the amplitude of the first contraction, whereas histamine and the antigen cause only a small increase in its amplitude, is evidence in support of the view that the Ca channels of rest and excitation differ in nature.

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