EFFECT OF LOCAL ANAPHYLAXIS ON ELECTRICAL ACTIVITY OF GUINEA PIG MYOCARDIAL FIBERS DURING INACTIVATION OF "FAST" SODIUM CHANNELS

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A preparation of the right auricle of the guinea pig atrium, preliminarily sensitized to egg albumin, was placed in Tyrode solution containing 20 mM KCl. Because of inactivation of the "fast" sodium channels of the membrane under these conditions stimulation of the preparation evoked only slow low-amplitude responses associated with activation of the "slow" sodium-calcium channels. These responses increased in amplitude and duration when egg albumin (2×10^{-4} g/ml), histamine (1×10^{-4} g/ml), and adrenalin (5×10^{-6} g/ml) were added to the solution. The results confirm the hypothesis of the leading role of "slow" sodium-calcium channels in the mechanism of the changes produced by cardiac anaphylaxis in the electrical activity of the myocardial fibers.

KEY WORDS: heart; anaphylaxis; ion transport; histamine; adrenalin.

The writers showed previously that the drug verapamil, which blocks "slow" sodium-potassium channels, reduces the heart rate and the duration of action potentials (APs) of the spontaneously contracting preparation during the development of anaphylaxis [5]. If the "fast" sodium channels of the membrane are inactivated by an excess of K^+ or blocked by tetrodotoxin, histamine causes an increase in the amplitude and duration of the electrical responses of the myocardial fibers, whereas a substance blocking the "slow" sodium-calcium channels (compound D-600) completely suppresses these responses [1].

On this basis the writers postulated that changes in electrical activity of the myocardial cells during local anaphylaxis and exposure to its probable mediator (histamine) are based on activation of the "slow" sodium-calcium channels of the excitable membrane.

The object of this investigation, part of a general investigation of the membrane mechanisms of immune reactions [1, 4-6], was to determine whether a local anaphylactic reaction can be obtained in myocardial fibers when the "fast" sodium channels are inactivated and, if so, to compare the changes in electrical activity during anaphylaxis and during the action of histamine.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated right auricle of the guinea pig atrium preliminarily sensitized to egg albumin by the method of Feigen and Prager [8]. Intracellular activity was recorded by glass electrodes filled with 2.5 M KCl. All the solutions used for perfusion were saturated with a gas mixture consisting of 96% O_2 and 4% CO_2. The temperature of the solutions was maintained throughout the experiment at between 36 and 37°C and the pH between 7.2 and 7.4. All the experiments were carried out on regularly contracting preparations. Each preparation was perfused initially with Tyrode solution of the following composition (in mM): NaCl 136.9, KCl 2.68, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, $NaH_2PO_4 \cdot 2H_2O$ 0.48, and

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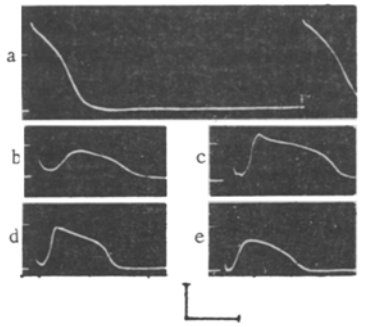

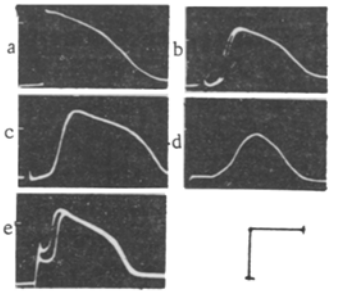

Fig. 1 Fig. 2

Fig. 1. Development of local anaphylactic reaction in partially depolarized guinea pig myocardial fibers: a) spreading APs of spontaneously contracting preparation of auricle in original Tyrode solution; b) slow response to electrical stimulation in solution with K^+ concentration raised to 20 mM; c-e) effect of egg albumin (2×10^{-4} g/ml) on amplitude and shape of slow response 2, 8, and 16 min, respectively, after its addition to "depolarizing" solution. Upper short line on left side of each frame shows zero potential level. Calibration: horizontally 100 msec, vertically 50 mV.

Fig. 2. Comparison of action of antigen and histamine (1×10^{-4} g/ml) on depolarized myocardial cells in same experiment: a) AP in Tyrode solution; b) slow response of cell on addition of 20 mM KCl to original solution; c, d) responses of cells 10 and 21 min, respectively, after addition of antigen (2×10^{-4} g/ml); e) increase in amplitude and duration of slow response in Tyrode solution containing histamine (1×10^{-4} g/ml). Calibration: horizontally 100 msec, vertically 50 mV.

glucose 5.6. The KCl concentration in the solution was then raised to 20 mM to depolarize the auricle. The remaining solutions were made up in this "depolarizing" solution: egg albumin (2×10^{-4} g/ml), histamine (1×10^{-4} g/ml), and adrenalin (5×10^{-6} g/ml). Evoked activity of the myocardial fibers was recorded under these conditions in response to stimulation by square pulses, 30-130 V in amplitude and 5-10 msec in duration. Silver electrodes applied to different ends of the preparation were used for stimulation. During exposure to each solution from 7 to 56 responses were recorded from one or several cells. The resting potential (RP) and the amplitude and duration of the AP at two levels ($\frac{1}{3}$ and $\frac{2}{3}$) of height of the pulse were measured in all frames. Effects were regarded as significant for which $P < 0.01$.

EXPERIMENTAL RESULTS AND DISCUSSION

In the original solution RP of the myocardial fibers varied from −68 to −87 mV and the amplitude of AP from 92 to 107 mV ($n = 63$). With an increase in the K^+ concentration to 20 mM the membrane was gradually depolarized and spontaneous activity of the preparation ceased. After perfusion for 5-15 min with the "depolarizing" solution RP gradually became stabilized and varied in different experiments from 38 ± 3.2 to 55.4 ± 2.1 mV. Electrical stimulation evoked slowly increasing low-amplitude responses.

On the addition of the antigen to the "depolarizing" solution as a rule considerable changes took place in the shape and duration of the slow responses. These changes quickly (in the course of 2-3 min) reached a maximum and then gradually declined. The effect was studied in 160 fibers (from 7 to 43 in one experiment). In four experiments addition of the antigen to the "depolarizing" solution was followed by an increase in both amplitude and duration of the response. In two experiments only the amplitude of the slow response, and in one only its duration, was increased. The maximal increase in amplitude was 150% and in duration 180%.

It will be clear from Fig. 1 (experiment carried out on one cell) that during perfusion of the preparation with the depolarizing solution the original spontaneous activity (Fig. 1a) stopped, after which slow

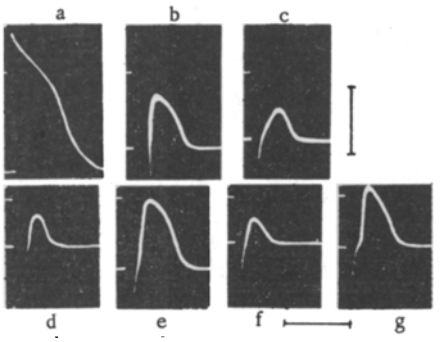

Fig. 3. Changes in shape and amplitude of slow responses of depolarized myocardial fibers produced by egg albumin (2×10^{-4} g/ml), adrenalin (5×10^{-6} g/ml), and histamine (1×10^{-4} g/ml): a) AP in Tyrode solution. Preparation rinsed with "depolarizing" solution (c, d, f) before addition of egg albumin (b), adrenalin (e), and histamine (g) to solution. Calibration: horizontally 100 msec, vertically 50 mV.

responses developed to stimulation in the partly depolarized fiber (1b). During the action of the antigen on the preparation (Fig. 1c) these responses first increased in both amplitude and duration, but then (Fig. 1e) gradually returned to their original low level in the course of 20-30 min.

In the experiment illustrated in Fig. 2, after the end of the reaction to the antigen (Fig. 2d) histamine was added to the solution (Fig. 2e). Comparison of the records in Fig. 2c and d shows that the changes in electrical activity of the myocardial cells under the influence of the antigen and histamine were externally very similar. In one experiment (Fig. 3) changes in electrical activity of partially depolarized myocardial cells evoked by antigen (Fig. 3c), adrenalin (Fig. 3e), and histamine (Fig. 3g) were compared. The changes in the amplitude and duration of the responses were similar in all three cases.

The temporal course of the reaction to the antigen in a solution with excess K^+ was exactly the same as the course of local cardiac anaphylaxis in Tyrode solution [2, 5, 8]. The effect of the antigen in both cases was expressed as an increase in the duration and amplitude of the responses. There are indications in the literature that in the course of the anaphylactic reaction, despite tachycardia, slight lengthening of the descending phase of the AP is observed [2, 8]. The changes in AP described in this paper were presumably true manifestations of anaphylaxis which are masked by tachycardia in a normal salt medium. In other words, membrane depolarization produced by elevation of the external K^+ concentration does not prevent the development of the anaphylactic reaction.

Responses of depolarized myocardial fibers are depressed by agents blocking "slow" sodium-calcium channels: verapamil and compound D-600 [14]. This suggests that the increase in amplitude and duration of the responses in the course of experimental anaphylaxis and during the action of histamine takes place through activation of "slow" sodium-calcium channels. Both histamine and the antigen evoked slight repolarization of the depolarized membrane in these experiments. This repolarization could weaken inactivation of the "fast" sodium channels and could thus increase the amplitude of the responses to the fast sodium current. However, this cannot explain the increase in duration of the response or, still less, the appearance of a plateau. The similarity between the effects of antigen and histamine, on the one hand, and that of adrenalin, which activates [7, 13] "slow" sodium-calcium channels, on the other hand, also confirms the role of the latter in the changes in electrical activity arising during anaphylaxis and exposure to histamine. In most animals, including the guinea pig [11], both Ca^{++} and Na^+ ions pass along these channels of myocardial fibers at the moment of AP generation [9, 10, 12]. The fact that an anaphylactic reaction can be obtained in the absence of Ca^{++} [3] thus does not conflict with the writers' conclusions regarding the role of "slow" sodium-calcium channels in the development of anaphylaxis.

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