# POSSIBLE ROLE OF SLOW SODIUM-CALCIUM CHANNELS IN THE MECHANISM OF CHANGES IN ELECTRICAL AND MECHANICAL ACTIVITY OF GUINEA PIG HEART CELLS IN LOCAL ANAPHYLAXIS (THE ISOPTINE EFFECTS)

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The local anaphylactic reaction and the effects of histamine during blocking of the slow sodium-calcium channels by isoptine were investigated in the spontaneously contracting oracle of the atrium of a guinea pig previously sensitized to egg albumin. Simultaneously with the intracellular recording of the potentials, isometric contractions of the preparation were recorded by means of a mechanotron. The investigation showed that egg albumin (0.2 mg/ml) and histamine (0.1 mg/ml) are antagonists of isoptine (2-16 mg/liter) as regards its effect on automatic contraction and duration of the action potential. It is postulated that this anaphylatic reaction is based on activation of the slow sodium-calcium channels in the surface membrane of the myocardial fibers.

KEY WORDS: heart; electrical activity; anaphylaxis.

It has recently been shown that the so-called slow sodium-calcium channels of an excitable membrane play an important role in the formation of the plateau of the action potential of myocardial cells, in the coupling of the excitation and contraction processes [3, 6, 7], and also, evidently, in the genesis of the automatic activity of pacemakers [1, 8, 10]. This provides a new basis for the elucidation of the mechanisms of the change in electrical and mechanical activity of the heart during exposure to various external agents and, in particular, for the study of the nature of local cardiac anaphylaxis.

In this investigation an approach was sought to the analysis of this phenomenon using the substance isoptine, a specific blocking agent of the slow sodium-calcium channels [5, 7].* The effect of isoptine was studied on the development of anaphylaxis and also on the effects of histamine, the probable mediator of this reaction [4].

## EXPERIMENTAL METHOD

Experiments were carried out on the isolated, spontaneously contracting auricle of the atrium of a guinea pig previously sensitized to egg albumin by the scheme described in [2]. Potentials were recorded by intracellular glass microelectrodes filled with 3 M KCl. Throughout the experiment the auricle was

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perfused with Tyrode solution of normal or modified composition, saturated with a gas mixture of 96% $O_2$ + 4% $CO_2$. The temperature of the solution was kept at between 34 and 36°C and the pH at 7.2. Contractions of the auricle were recorded under isometric conditions by means of a mechanotron.

## EXPERIMENTAL RESULTS AND DISCUSSION

In seven control experiments the addition of egg albumin (0.2 mg/ml) to the perfusion fluid induced a typical anaphylactic reaction of the sensitized preparation. The frequency of its spontaneous activity was increased (by 24-98%) and the contractions were strengthened by 1.5-2 times. Similar changes in the electrical and mechanical activity of the auricle (both sensitized and normal) were induced by histamine (0.1 mg/ml). However, whereas during the anaphylactic reaction these effects were transient in character, in the case of histamine the high frequency and strength of the contractions persisted steadily during the 30-40 min of action of the substance.

Isoptine (12 experiments) had a multilateral action on the activity of the auricle. In concentrations of isoptine of 2-4 mg/liter the frequency of the spontaneous activity was reduced, whereas in a concentration of 8-16 mg/liter this activity ceased completely. In two experiments the decrease in frequency was preceded by a short increase. Changes in the action potential (AP) were characteristic of isoptine. The maximal steepness of rise of the AP was increased by 25-50% and its duration (especially in the plateau phase) was considerably shortened; in place of the former plateau, a clearly distinguishable notch appeared (Fig. 2b, d). In every case under the influence of isoptine uncoupling of excitation and contraction took place: contraction disappeared almost completely during the first minutes of action of isoptine in concentrations of 4-8 mg/liter. All these results are in good agreement in principle with those obtained by other workers [5].

Egg albumin (10 experiments), administered after isoptine, led to an increase in the frequency of contraction of the sensitized auricle characteristic of the anaphylactic reaction. However, the frequency in this case only reached the original values observed in the preparation before administration of isoptine. If isoptine in a concentration of 8 mg/liter caused arrest of the auricle, the atomatic contractions were resumed under the influence of egg albumin.

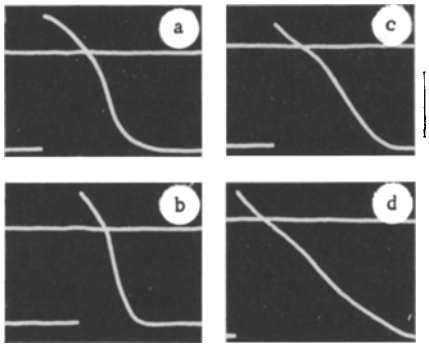


Fig. 1. Effect of egg albumin and histamine on action potential of spontaneously contracting myocardial fibers of a sensitized guinea pig: a) preparation in Tyrode solution; b) effect of isoptine, 4 mg/ml liter; c) effect of egg albumin in a dose of 0.2 mg/ml after previous administration of isoptine; d) repeated lengthening of plateau of AP on addition of histamine (0.1 mg/ml) to solution with isoptine after disappearance of effect of egg albumin. Mean frequency of excitation of preparation: a) 120; b) 70; c) unstable rhythm; d) 60 (here and in Figs. 2 and 3, cycles last 1 min). Calibration: vertically 50 mV; horizontally 50 msec. Experiment on April 19, 1973.

Under the influence of egg albumin the APs of the myocardial cells underwent characteristic changes. As will be clear from Fig. 1c, during the anaphylactic reaction induced by egg albumin the AP shortened by isoptine was clearly lengthened, both on account of the plateau and as a result of prolongation of the final phase of repolarization. As a rule this lengthening of the AP was observed only during the first few minutes of development of the anaphylactic reaction, and later the effect of isoptine took priority and the AP was again shortened (Fig. 2c and d). The addition of histamine (0.1 mg/ml) to the solution again lengthened the AP (Fig. 1d). Egg albumin did not abolish the uncoupling of excitation and contraction caused by isoptine. However, if the contraction was not completely inhibited, it was strengthened by about 50% during the anaphylactic reaction.

Like egg albumin, histamine (five experiments), given after previous administration of isoptine, caused an increase in the discharge frequency. This increase was usually of short duration and was followed by a fresh decrease. The AP plateau was distinctly lengthened by histamine (Fig. 3c). If isoptine (8 mg/liter) suppressed the automatic contractions, histamine restored them but the frequency of excitation under these circumstances was low.

The effects of egg albumin and histamine were compared with the effect of an increase in the Ca ion concentration in the medium. A fivefold increase in the Ca concentration of the solution containing isoptine caused lengthening of the shortened plateau; the contractile activity not

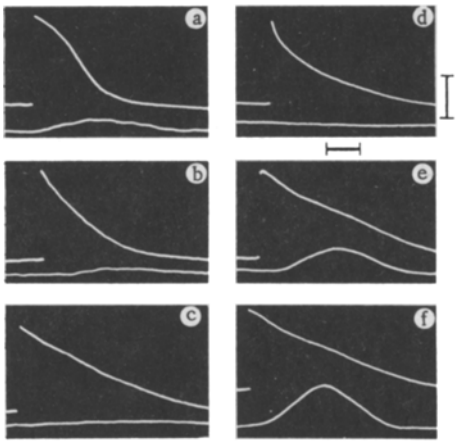


Fig. 2

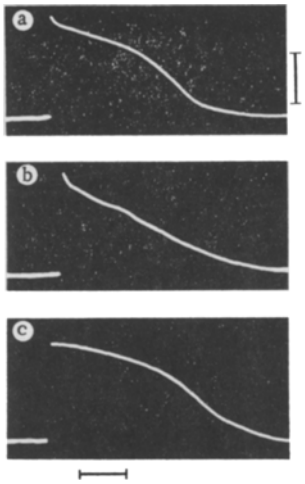


Fig. 3

Fig. 2. Effect of egg albumin (a-d) and of increase in Ca-ion concentration (e-f) after previous administration of isoptine: a) AP in Tyrode solution; b) the same after addition of isoptine (8 mg/liter) to the solution; c, d) 6 and 14 min of anaphylactic reaction; e, f) 3 and 8 min after fivefold increase in external Ca concentration. Mean frequency: a) 180; b) 100; c) 120; e, f) 60. Calibration: vertically 50 mV; horizontally 25 msec. Experiment on July 12, 1973.

Fig. 3. Effect of histamine after previous administration of isoptine: a) AP in Tyrode solution; b) the same after addition of isoptine (8 mg/liter); c) after 9 min of action of histamine (0.1 mg/ml). Mean frequency: a) 180, b) preparation did not contract spontaneously, c) 40. Calibration: vertically 50 mV; horizontally 25 msec. Experiment on April 20, 1973.

only was restored but was considerably increased above its initial level (Fig. 2e, f).

There are good grounds for considering that local anaphylaxis arises as a result of the liberation of histamine from the mast cells present in heart tissue through the influence of specific interaction between antigen (in this case, egg albumin) and antibody fixed on their membrane [4]. On the basis of modern views on the mechanisms of action potential generation in the myocardial cells and on the connection between excitation and contraction it is very tempting to suggest that the effects of histamine are linked with activation of the slow sodium-calcium channels in the membranes of these cells. Strengthening of the slow inward current in the cells of the pacemaker increases the rate of interspike depolarization (pacemaker potential) and also, possibly, lowers the critical potential [2]. Strengthening of the inward flow of Ca ions in the cells of the auricle during the AP, on the other hand, strengthens their contraction. According to this hypothesis histamine acts as an antagonist of isoptine, for the latter induces directly opposite changes in the electrical activity of the myocardial cells – slowing of the rhythm and weakening of their contraction. However, during the combined administration of isoptine and histamine or isoptine and egg albumin, antagonism between them is manifested only as regards their effect on the frequency and duration of the action potential: histamine and egg albumin increase the rhythm and prolong the AP when shortened by isoptine. In this respect the effects of histamine differ significantly from the effects of Ca ions, which restore both the AP plateau and the myocardial contractions.

The reasons for these differences are not yet clear. Isoptine perhaps blocks the calcium channels not only in the surface membrane but also in the membranes of the sarcotubular system. Competition between histamine and isoptine takes place, however, only on the surface membrane. Further investigations will show how far the hypotheses suggested above correspond to the true relationships.

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