EFFECT OF HISTAMINE ON ELECTRICAL AND MECHANICAL ACTIVITY OF THE MYOCARDIAL FIBERS OF THE GUINEA PIG HEART AFTER BLOCKING OF THE FAST SODIUM CHANNELS

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The effect of histamine on electrical (recorded intracellularly) and mechanical (isometric contraction) activity of the auricular myocardial fibers of the guinea pig atrium was investigated after preliminary blocking of the fast sodium channels by prolonged depolarization with K^+ ions (20 mM) or with tetrodotoxin (3.2×10^{-7}-2.0×10^{-6} g/ml). Slowly increasing gradual responses to short stimuli were found to appear in the depolarized fibers. Histamine (10 μg/ml) increased the amplitude and duration of these responses, and at the same time the amplitude of the contractions of the preparation also increased. Compound D-600 (1 mg/liter), which specifically blocks calcium channels, inhibited the slow responses and mechanical activity of the preparation. After administration of tetrodotoxin alone or in combination with KCl, histamine also had a potentiating action of its own. It is concluded that histamine activates the slow sodium–calcium channels of the myocardial excitable membrane.

KEY WORKS: myocardium; effects of histamine; slow and fast membranes.

In a previous investigation [2] on the spontaneously contracting auricle of the guinea pig heart [2] it was postulated that changes in the electrical activity of the myocardial cells during experimental cardiac anaphylaxis [1] and during the action of histamine, the probable mediator of this response [3], are due to activation of the so-called slow sodium–calcium channels of the excitable membrane. To test this hypothesis, in the present investigation the effects of histamine were studied after preliminary blocking of the fast sodium channels. This was done either by prolonged potassium depolarization of the membrane (to about 50 mV), inducing almost total sodium inactivation [5-7], or with the aid of tetrodotoxin (TTX; 3.2×10^{-7}-2.0×10^{-6} g/ml), or as a result of the combined administration of these factors. The preparation D-600 [4]* was used to block the slow channels.

EXPERIMENTAL METHOD

Experiments were carried out on the right (spontaneously active) and left auricles of the guinea pig heart. Electrical activity was recorded with glass electrodes filled with 2.5 M KCl solution and isometric contractions with the aid of a mechanotron. The animals were immobilized by a blow on the head, the chest was opened, and the heart removed and placed in warm Tyrode's solution saturated with a mixture of 95% O_2 and 5% CO_2. After removal of the last traces of blood the auricles were cut off and placed in the working chamber through which a current of Tyrode's solution flowed. One of the auricles (depending on the aim) was then stretched on the floor of the chamber under isometric conditions. One end of the object was attached to the rod of the mechanotron and the other fixed by means of a hook secured to a magnetic sup-

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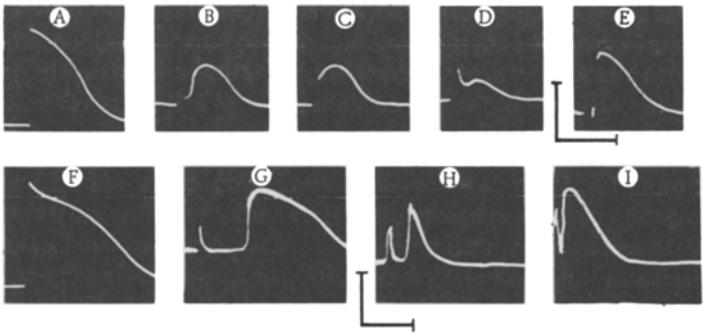

Fig. 1. Effect of TTX, the preparation D-600, and histamine on slow electrical responses of auricular cells of the guinea pig atrium: A) spreading action potential of the spontaneously contracting auricle in Tyrode's solution of the original composition; B) spontaneous activity arrested by the addition of 20 mM KCl to the Tyrode's solution. Slow response evoked by a short (5 msec) electrical stimulus; C) the same, but after addition of TTX (2×10^{-6} g/ml) to the solution; D) combined action of TTX and D-600 (1×10^{-6} g/ml); E) rinsing the preparation with Tyrode's solution; F and G) the same as A and B respectively; H) addition of 2×10^{-6} g/ml TTX to the KCl solution; I) the same, but with the addition of histamine (1×10^{-6} g/ml). Calibration: vertically 50 mV, horizontally 50 msec.

port. The composition of the Tyrode's solution was as follows (in mM): NaCl 136.9, KCl 2.68, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, $NaH_2PO_4 \cdot 2H_2O$ 0.42, and glucose 5.6. For depolarizing the membrane the KCl concentration in the Tyrode's solution was raised to 20 mM. The preparations were stimulated with square pulses 15-150 V in amplitude and 5-10 msec in duration by means of silver electrodes applied to different ends of the preparation. During the experiments the temperature was maintained at 35-37°C and the pH at 7.2-7.4.

EXPERIMENTAL RESULTS AND DISCUSSION

Several investigators have shown [6-8] that a gradual electrical response (GR) connected with activation of the slow sodium–calcium channels appears in fibers of the right ventricle of the sheep's and cat's heart, depolarized by potassium ions, to the application of a short stimulus. The present experiments confirmed this conclusion. Increasing the KCl concentration in the Tyrode's solution to 20 mM (depolarizing solution) lowered the resting potential from -78.02 ± 7.5 to -50.2 ± 9.7 mV ($n = 103$ fibers; $P < 0.01$). The spontaneous activity of the fibers was arrested. GRs similar to those illustrated in Fig. 1B appeared in response to electrical stimulation. These responses were relatively insensitive to 1×10^{-6} g/ml TTX, and they were virtually completely suppressed by the preparation D-600 (1 mg/liter).

The addition of histamine (10 μg/ml) to the depolarizing solution in all experiments (15) without exception led to an increase in amplitude and a marked increase in the duration of the GR. In some cases the GRs lost their gradual character and acquired a long and distinct plateau (Fig. 2C). This potentiating action of histamine was particularly well marked during the first 5-10 min after its administration, and later it weakened somewhat and became stabilized.

In 13 experiments histamine reduced the membrane depolarization evoked by KCl only very slightly (the resting potential rose to -57.7 ± 12.3 mV, $n = 83$, $P < 0.01$), but no correlation could be found between the intensity of the potentiating action of histamine and changes in the resting potential. Sometimes under the influence of histamine (three cases) the spontaneous activity of the preparation was briefly restored.

As a rule (8 of 10 experiments) depolarization of the membrane by K^+ ions led to a considerable decrease in amplitude of the contractions. Only in two cases was the opposite effect observed – the contractions were strengthened. In all experiments histamine increased the amplitude of the contractions of the preparation (Fig. 2C and D). Clearly defined antagonism between histamine and the preparation D-600 was observed as regards their effect on electrical and mechanical activity of the fibers. Whereas in the absence

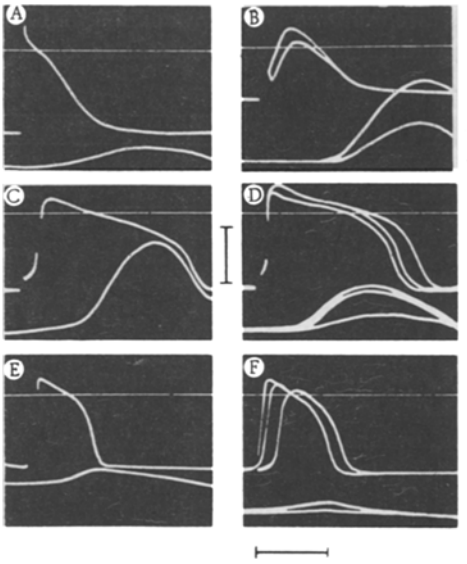

Fig. 2. Potentiating effect of histamine on electrical and mechanical activity of myocardial cells: A) action potential and isometric contraction of spontaneously active auricle of atrium in Tyrode's solution (frequency 180 cycles/min); B) slow gradual responses to stimulation in depolarizing KCl solution (20 mM). Superposed curves obtained with two strengths of stimulation (40 and 60 conventional units respectively); C, D) the same, after the addition of histamine (1×10^{-5} g/ml) to the solution, effects after 1 and 5 min respectively (strength of stimulation: C, 40 conventional units; D, 40, 60, and 80 conventional units); E and F) combined action of histamine and D-600 (10^{-6} g/ml) 4 and 8 min later respectively (strength of stimulation: E, 40 conventional units; F, 40, 60, and 80 conventional units). Calibration: vertically 50 mV, 25 mg; horizontally, 50 msec.

of histamine, D-600 virtually completely suppressed the electrical response (Fig. 1D), if the two substances were given together, this response persisted (Fig. 2E and F).

In five experiments the action of histamine was investigated after administration of TTX. In three of these experiments histamine was used during depolarization of the membrane by KCl and in two experiments in the presence of the usual concentration of K^+ ions. As was stated above, TTX in a concentration of 1×10^{-6} g/ml in depolarizing solution caused virtually no change in the GRs (Fig. 1C). When the TTX concentration was increased to 2×10^{-6} g/ml, the duration of the response after 10-15 min was appreciably reduced (Fig. 1H). The addition of histamine to the solution caused a marked increase in the duration and amplitude of the GRs (Fig. 1I).

In normal Tyrode's solution, TTX in a concentration of 2×10^{-6} g/ml caused hyperpolarization of the membrane of the fibers (the resting potential was increased to -105.6 ± 6.0 mV, $n = 14$; $P < 0.01$). The spontaneous activity of the auricle persisted but the conduction of electrical impulses between separate fibers of the myocardium was dissociated. The leading edge, amplitude, and duration of the action potentials were appreciably lowered. Histamine increased the duration of the electrical responses on account of the prolongation of the descending phase of the action potential. The leading edge and amplitude of the responses were lowered, probably because of the continued action of the TTX. Parallel with lengthening of the electrical responses, the amplitude of the contractions was increased (Fig. 3).

The writers showed previously [2] that histamine and the substance Isoptin, blocking slow sodium–calcium channels, are antagonists as regards their effect on the rhythm of spontaneous activity and on the duration of the plateau of action potentials of the auricular myocardial fibers of the quinea pig atrium: Isoptin slows the rhythm and greatly shortens the duration of the descending phase of the action potential by "cutting off" the plateau. Histamine (10 μg/ml) if added to the solution containing Isoptin, restored the spontaneous rhythm (or increased its frequency if it had not been completely suppressed) and considerably prolonged the shortened action potential, thereby restoring the plateau. Similar changes in electrical activity of the atrial auricle were obtained if an antigen (egg albumin) to which the guinea pig had previously been sensitized, was given during the period of action of Isoptin. During the local anaphylactic reaction the spontaneous activity of the auricle was resumed or its frequency was increased, and the descending phase of the action potential was lengthened. However, these experiments were carried out during a time of normal functioning of the fast sodium channels and, for that reason, they are open to a different interpretation. In the present investigation, in order to test the hypothesis expressed above, the effects of histamine were tested after preliminary inactivation of the sodium channels or their pharmacological blocking by TTX. We have seen that the action of histamine not only would not weaken under these conditions, but on the contrary, it became particularly clear. The gradual responses connected with the functioning of the slow channels were increased by histamine, and they were very considerably prolonged as the result of plateau formation. Meanwhile the contraction of the auricle was strengthened. Distinct antagonism was found between the effects of histamine and those of the substances D-600 (the methoxy derivative of Isoptin), blocking the slow channels. All these observations confirm the view that the slow channels play a role in the mechanism of the changes in electrical activity of the myocardial cells under the influence of histamine. Preliminary experiments carried out

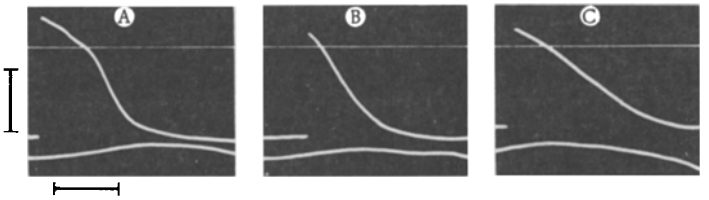

Fig. 3. Action of histamine after preliminary administration of TTX: A-C) spreading action potentials and isometric contraction of spontaneously active auricle of the atrium (frequency 210 cycles/min): A) in Tyrode's solution; B) addition of TTX (2×10^{-6} g/ml) to the solution, frequency 80 cycles/min; C) combined action of TTX (2×10^{-6} g/ml) and histamine (1×10^{-5} g/ml), frequency 80 cycles/min. Calibration: vertically, 50 mV, 125 mg; horizontally, 50 msec.

on guinea pigs sensitized to egg albumin showed that blocking the fast sodium channels by TTX and (or) of potassium depolarization does not prevent the development of a local anaphylactic reaction which, in the case in question, is expressed as the strengthening and lengthening of the slow responses. In other words, here also the effects of histamine and the antigen coincide.

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