MECHANICAL OSCILLATIONS IN THE GUINEA PIG AURICLE DURING COOLING

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In some cases mechanical activity of isolated fragments of myocardium from warm-blooded animals is accompanied by periodic damped oscillations of isometric strain or isotonic contractions, which develop immediately after the end of contraction evoked by an external stimulus (mechanical oscillations). The conditions for appearance of mechanical oscillations in myocardial cells are somewhat variable. In the papillary muscles of the guinea pig heart "postcontractions" have been recorded in medium with an increased Ca^{++} ion concentration, in response to an increased frequency of stimulation, and under the influence of dihydroouabain. "Postcontractions" developed against the background of oscillations of membrane potential [6]. In preparations of guinea pig auricle mechanical oscillations have been found, accompanied by electrical oscillations during lowering of the temperature of the perfusion solution to 20-10°C. However, other workers [3] found no such changes of membrane potential during the development of mechanical oscillations either in preparations of the

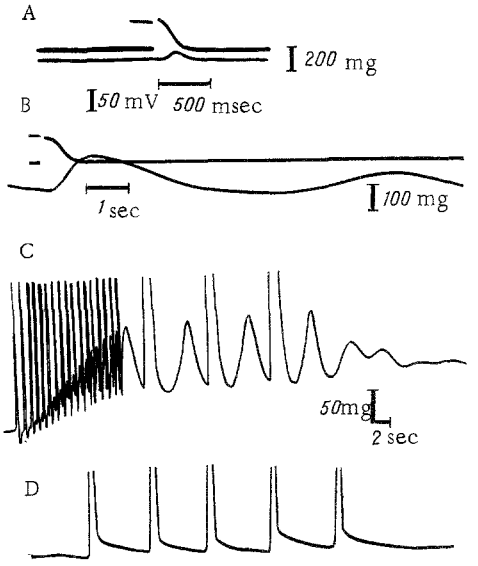

Fig. 1. Absence of oscillations of membrane potential during development of first half-period of mechanical oscillations. A) Action potential and contraction in Tyrode solution (33°C), B) the same during rapid lowering of the temperature of the solution to 10°C, C) regular contractions and mechanical oscillations between them, D) reaction of mechanotron–preparation system to holding mechanotron rod.

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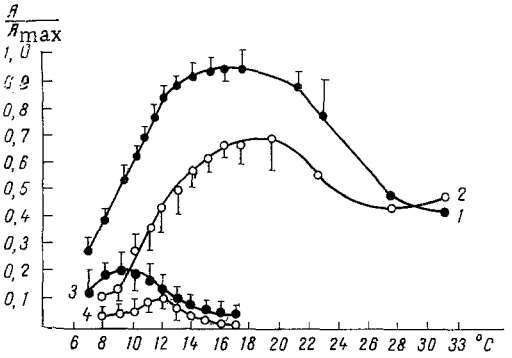

Fig. 2. Dependence of development of contractions and mechanical oscillations on temperature. 1 and 3) Contractions and oscillations, respectively, in Tyrode solution ($CaCl_2$ concentration 18 mM), 2 and 4) the same, after addition of 1 mM caffeine to the solution. A_{max}) Maximal amplitude of contraction or of first half-period of oscillations. A) Amplitude of contraction and oscillation at that temperature.

auricle or in papillary muscles of guinea pig heart when the temperature of the perfusion solution was lowered to 14-10°C. Brief oscillations of mechanical activity (for 10-15 min) were observed in the papillary muscles of the cat's heart in Tyrode solution with a reduced Na^+ concentration and an increased K^+ concentration [5]. Mechanical oscillations have been recorded in single heart muscle fibers isolated from the rat ventricle, after removal of the surface membrane, in response to an increase in the external Ca^{++} concentration [1].

The object of this investigation, conducted on the guinea pig auricle, was to study the temperature dependence (within the range 17-7°C) of development of mechanical oscillations arising spontaneously after contraction to a stimulus.

EXPERIMENTAL METHOD

The guinea pig auricle served as the test object. Preparations were placed in a perfusion chamber through which flowed Tyrode solution (composition in mM: NaCl 136.9, KCl 2.68, $NaHCO_3$ 11.95, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $CaCl_2 \cdot 2H_2O$ 1.8, glucose 5.6), saturated with carbogen (95% O_2 + 5% Co_2), pH 7.2-7.4. The normal temperature in the chamber was maintained at 33-34°C. For the 30 min before the experiment began the preparation was stimulated by supraliminal pulses with a duration of 5-10 msec and a frequency of 1 Hz. Stimulation was applied through two silver electrodes located in the chamber along the preparation. Contractions were recorded isometrically by the 6MKhlS mechanotron. Contractions were recorded on film from the screen of a type S1-18 oscilloscope. The preparation was cooled by lowering the temperature of the perfusion solution with a microrefrigerator. The working temperature range was 33-7°C. During the study of mechanical contractions the preparation was stimulated by supraliminal pulses with a duration of 20-70 msec and a frequency of 0.1 Hz. Intracellular potentials were measured by means of "floating" glass microelectrodes filled with 2.5 M KCl (resistance of the tip 10-20 mΩ).

EXPERIMENTAL RESULTS

Cooling the myocardium of the guinea pig auricle to 17-7°C led to the development of spontaneous mechanical oscillations, unaccompanied by any changes in membrane potential of the myocardial fibers, immediately after the end of the next in the regular series of contractions. A record of one of the 10 experiments of this series is illustrated in Fig. 1A and B. It will be clear from Fig. 1 that a rapid fall of temperature (in the course of 1 min) from 33°C (frame A) to 10°C (frame B) led to an increase in duration of the action potential (AP) and to the appearance of a well-defined plateau. Under these circumstances the initial resting potential fell a little. After the end of contraction evoked by the AP, the first half-period of mechanical oscillations appeared. As a rule, under steady-

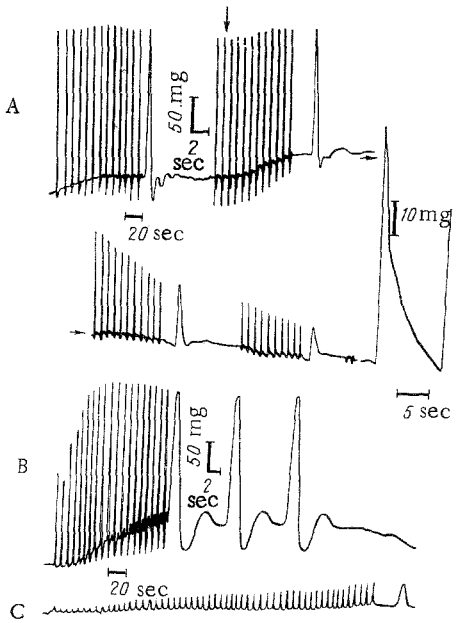

Fig. 3. Disappearance of mechanical oscillations in solution containing 10 mM caffeine or 20 mM KCl (effects of caffeine and excess of KCl). A) Contractions and oscillations in Tyrode solution (10°C), arrow indicates time of addition of caffeine, B) contractions and oscillations in Tyrode solution (10°C), C) disappearance of oscillations on addition of 20 mM KCl.

state conditions, in solution at a temperature of 10°C, 0.5-1 period of mechanical oscillations was able to develop between two consecutive contractions in the regular series. Immediately after the appearance of the next contraction, when stimulation of the preparation stopped, mechanical oscillations of damped character appeared (Fig. 1C). The appearance of the oscillations was not connected with the presence of elastic elements in the mechanotron preparation system. As Fig. 1D shows, no mechanical oscillations appeared when the rod of the mechanotron, rigidly connected to the preparation, was simply held.

The amplitude and number of oscillations depended on the Ca^{++} concentration in the Tyrode solution. With a tenfold rise in the external Ca^{++} concentration the amplitude of the oscillations increased sharply and their number reached eight; the period of oscillations was correspondingly reduced and the amplitude of the main contractions also increased. The same effects were observed when the 50% NaCl was replaced by isotonic sucrose solution. In potassium-free solution and in solution containing compound D-600, which blocks slow Na,Ca-channels, the amplitude of the mechanical oscillations and of the main contractions was reduced.

Dependence of the relative amplitude of contractions of the preparation (curve 1) in the steady-state and of the first half-period of oscillations (curve 3) in Tyrode solution with 18.8 mM $CaCl_2$ on temperature is shown in Fig. 2. Clearly the amplitude of contractions of the preparation increased as the temperature was lowered from 33 to 16°C (nine experiments). Mechanical oscillations appeared at 17°C. Their amplitude increased with further cooling of the preparation to reach a maximum at 9°C. At lower temperatures the amplitude of the oscillations fell. The damping time of the oscillations (τ_{damp}) was determined; this is the time during which the amplitude of the oscillations was reduced by 2.7 times. Within the range from 12 to 8°C there was no significant change in τ_{damp}. The period of the oscillations increased from 2.87 ± 0.4 (14°C) to 5.8 ± 0.5 sec (7°C). In a special series of 10 experiments substances significantly exhausting the Ca^{++} reserves in the sarcoplasmic reticulum (SR) of the myocardial fibers were used. The addition of 1 mM caffeine to the solution caused a decrease in the amplitude of contractions (Fig. 2, curve 2) and oscillations (curve 4) within the range from 29 to 7°C, and the maximum of the oscillations was shifted from 9°C in Tyrode solution to 12°C in solution with caffeine. An increase in the

caffeine concentration in the solution to 10 mM led to a sharp decrease in the amplitude of contractions in the regular series and to complete disappearance of the mechanical oscillations (Fig. 3A). Tyrode solution with an increased KCl concentration (20 mM) had a similar action (Fig. 3B, C) on the regular contractions and spontaneous mechanical oscillations. In both cases the temperature of the solutions was 10°C.

It can now be regarded as firmly established that the force of contraction of a muscle fiber, other conditions being the same, is determined by the concentration of free Ca^{++} ions in the myoplasm in the region of the myofibrils [2]. The amplitude of contractions of the preparation was found to increase progressively as the temperature was lowered from 29 to 17°C, evidently because of an increase in the Ca^{++} concentration near the myofibrils of the heart muscle cells after the end of each successive contraction in the regular series. The amplitude of the contractions reached a maximum at 17°C, and the first mechanical oscillations appeared at that same temperature. The question of the progressive decrease in the amplitude of contractions at temperatures below 17°C has not yet been answered, but coincidence of the maximum of the contractions and the temperature threshold of development of the oscillations can be regarded as evidence that both the time of appearance of the oscillations (at 17°C) and the increase in their amplitude as the temperature is lowered further are probably connected with the fact that the residual calcium in the myoplasm cannot be pumped out by the longitudinal system of the SR of the heart cells, whose sequestering ability is depressed at the temperatures studied. The calcium remaining in the myoplasm evokes a regenerative release of calcium from the lateral cisterns of SR. Evidence that it is SR which is responsible for generation of the mechanical oscillations is given by the fact that treatment with substances (10 mM caffeine and 20 mM KCl) leading to exhaustion of the Ca^{++} reserves in SR caused complete disappearance of the oscillations although the contractions remained as before. There are also indications in the literature that SR of the myocardial fibers of warm-blooded animals is the sole source responsible for the appearance of mechanical oscillations [2]. Damping of the oscillations is probably attributable toleaking of Ca^{++} ions from the myoplasm of the fibers. Since τ_{damp} is virtually independent of temperature, it can be postulated that leaking of Ca^{++} ions at low temperatures is a passive process.

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