EFFECT OF CHANGES IN Na, K, AND Ca ION CONCENTRATIONS IN THE MEDIUM ON FREQUENCY – STRENGTH OF CONTRACTION RELATIONS IN THE PATHOLOGICALLY CHANGED MYOCARDIUM

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The following criteria of myocardial insufficiency were studied in fragments of human atrial myocardium (auricle) removed during operative correction of diseased heart valves: 1) the decrease in amplitude of contractions in a rhythmic series despite a frequency of stimulation of 1-3 Hz (the normal myocardium is characterized by an increase in the amplitude of contraction – Bowditch's positive treppe); 2) the monophasic character of the frequency–strength curve (in the normal myocardium the curve has three phases); 3) the absence of positive or appearance of a negative inotropic effect in response to a decrease in the external Na concentration or removal of K ions from the solution. The results suggest that myocardial failure of the cardiac cells of the affected heart is based on a disturbance of Ca ion transport through the surface membrane and the reduced ability of the sarcoplasmic reticulum to retain Ca ions.

KEY WORDS: human myocardium; contraction; criteria of insufficiency; Na, K, and Ca ions.

Investigation of the mechanisms of the positive and negative inotropic action of various chemical agents on the myocardial cells of the pathologically changed heart is interesting both to shed light on the nature of cardiac failure and in order to develop effective methods of its treatment. The strength of cardiac contraction can be considerably increased, as we know, by changing the ionic composition of the medium. For instance, a decrease in the external concentration of Na ions (not below 30% of normal), the partial or total removal of K ions, and a two- to fivefold increase in the Ca concentration caused a distinct positive inotropic effect in the heart of warm-blooded animals [2]. Considering the results of previous investigations on the human myocardium [6, 10], in the present study special attention was devoted to the effect of the ionic composition of the medium on frequency–strength relations during contraction of the pathologically changed myocardium.

The object of the investigation, which was conducted on fragments of atrial myocardium removed from patients during reconstructive operations on decompensated valvular defects of the heart, was to determine to what extent ability to develop a positive inotropic reaction to an increase in the rhythm of stimulation and (or) a change in the concentrations of Na, K, and Ca ions in the medium can be used to indicate the state of the cells of the patient's myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on fragments of the auricles from the atria of 40 patients. After removal the auricle (right – 22, left – 18) was immersed in oxygenated Tyrode's solution at room temperature. The time from taking the tissue during the operation until its arrival in the laboratory was 15-20 min. Trabeculae 4-5 mm long, 1-2 mm wide, and not more than 1 mm thick were then removed from the auricle. The trabeculae thus removed were placed in a perfusion chamber through which Tyrode's solution, enriched with carbogen (95% O_2 and 5% CO_2) and heated to 35-36°C, flowed. For 1 h before the beginning of the investigation the trabuculae were stimulated at a frequency of 0.5 Hz. Two silver electrodes, located at the edges of the preparation, were used for stimulation. Above-threshold stimuli 5-10 msec in duration were applied. The duration of individual series of stimulation varied from 20-30 sec to 1-2 min. The interval between series was 5 min. Contractions were recorded under isometric conditions by means of the 6MKh1S mechanotron. The frequency

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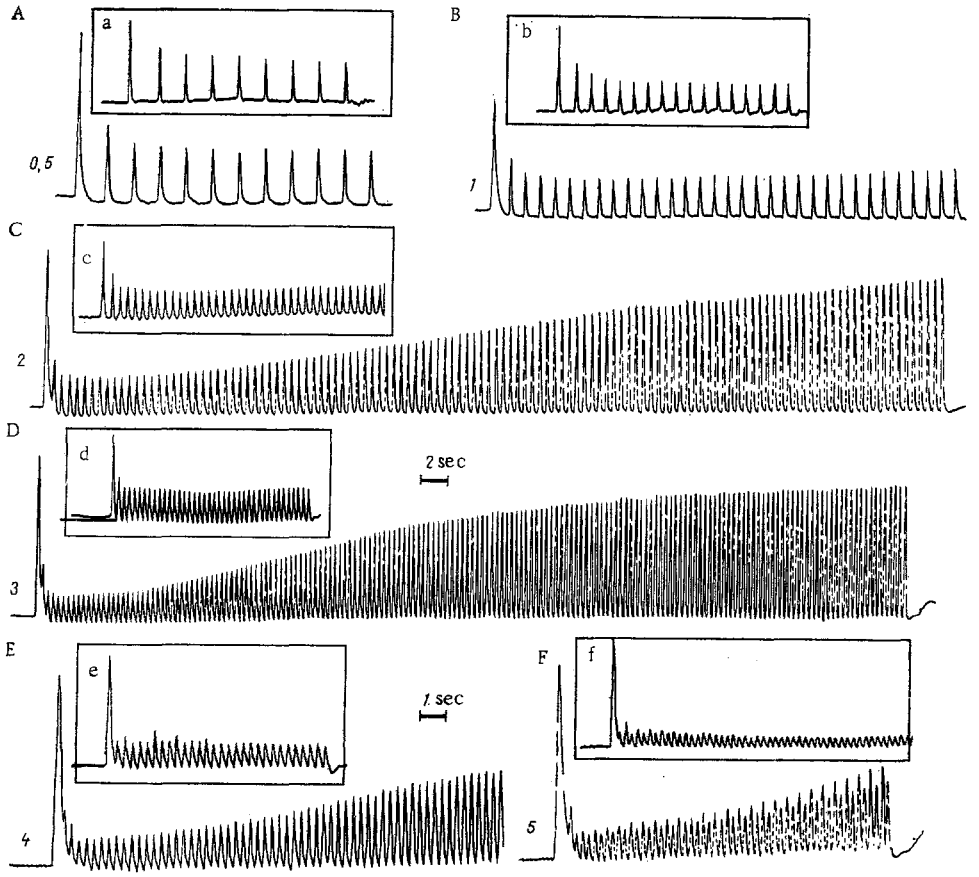

Fig. 1. Two variants of responses of atrial myocardium to repetitive stimulation: a-f) preparation from first group, negative Bowditch's ladder; A-F) preparation from second group, positive Bowditch's ladder. Numbers before each record on left denote frequency of stimulation, in Hz. Time calibration for A-D 2 sec, for E, F 1 sec.

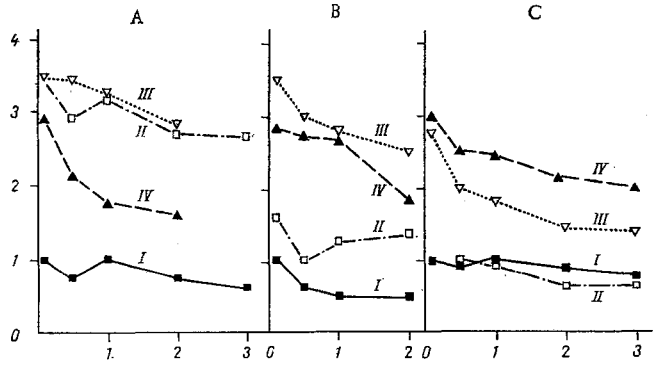

Fig. 2. Changes in frequency–strength curves in Tyrode's solution with change in concentrations of Na, K, and Ca ions. A, B, C) Separate experiments. A and C) Preparations of normal myocardium (frequency–strength curves in Tyrode's solution had ill-defined triphasic shape). B) Preparation of failing myocardium (in original solution frequency–strength curve monophasic). I) Ordinary Tyrode solution; II) 60% Na solution; III) solution with twofold increase in Ca concentration; IV) potassium-free Tyrode solution. Abscissa, frequency of contractions (in Hz); ordinate, amplitude of contractions (in conventional units).

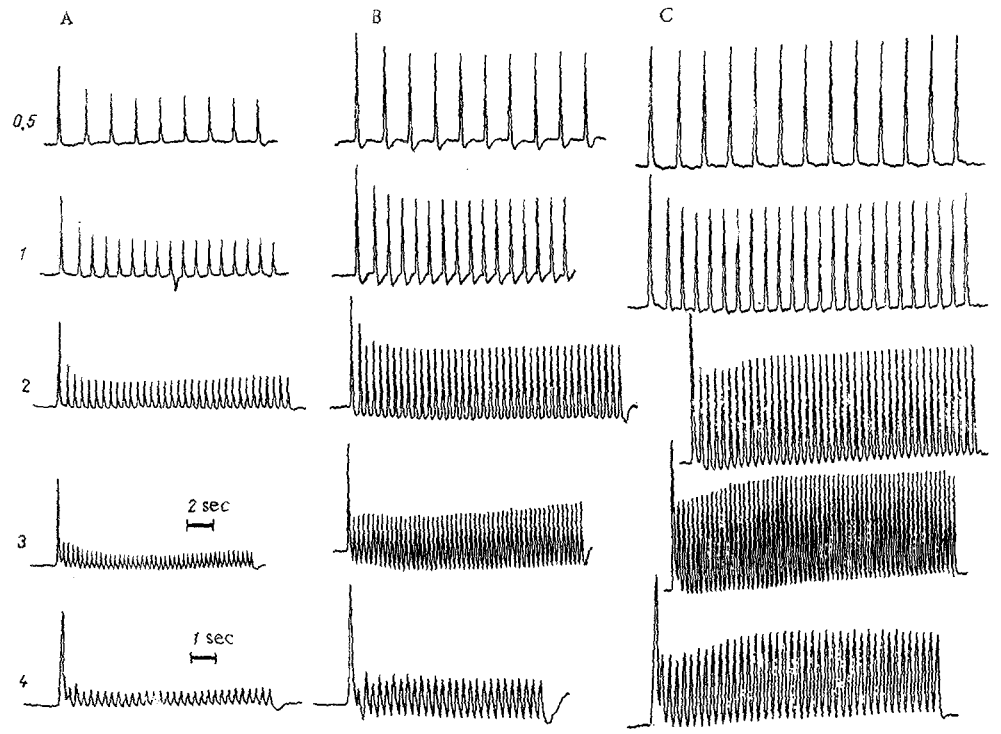

Fig. 3. Changes in amplitude of first and subsequent contractions of rhythmic series in potassium-free and high-calcium solutions. A) Negative Bowditch's treppe in ordinary Tyrode's solution; B) change in contractions in K-free solution; C) same after twofold increase in Ca concentration. Remainder of legend as in Fig. 1.

of stimulation was varied in the course of the experiment from 0.5 to 5 Hz. Contractions were recorded on the Mingograph-81. The Tyrode's solution used had the following composition (in mM): NaCl 131, KCl 4.5, $NaHCO_3$ 11, $NaH_2PO_4 \cdot 2H_2O$ 0.6, $MgCl_2 \cdot 6H_2O$ 0.25, $CaCl_2 \cdot 2H_2O$ 2.16, and glucose 11. The concentration of Na ions in the solution was reduced by 40% and replaced by sucrose in the isosmotic concentration. In the case of complete removal of K ions from the solution or a twofold increase in its Ca ion concentration, no osmotic compensation was carried out.

EXPERIMENTAL RESULTS

All the preparations tested were divided into two main functional groups. The first group (17 preparations) had frequency-contraction relations that differed significantly from those observable in the normal myocardium of warm-blooded animals. The differences were that the increase in amplitude of the contractions of the repetitively stimulated preparations was very slight at frequencies of stimulation of 1-3 Hz (6 experiments) or was absent altogether – there was a steady decrease in the amplitude of contractions in a rhythmic series (11 experiments) (Fig. 1a-f). In the normal myocardium of warm-blooded animals and man the amplitude of contractions measured at the level of the plateau of Bowditch's treppe rises with an increase in the frequency of stimulation from 0.5 to 2-3 Hz, but at higher frequencies of stimulation the amplitude falls (Fig. 1A-F). As a result, the frequency–strength relationship (f–F) can be represented as a curve with three phases [6, 8, 12]. Since the increase in amplitude of the contractions in a rhythmic series was negligible or absent in the first group of preparations, the f–F curve was monophasic in character. The monophasic character of the f–F curve is usually regarded as evidence of myocardial insufficiency [6, 9]. With a decrease in the external Na ion concentration by 40%, a positive inotropic effect, characteristic of the normal myocardium of warm-blooded animals, developed in only 7 of the 17 preparations of the first group. In the other 11 preparations in a solution with low sodium concentration the amplitude of contractions either was unchanged or somewhat reduced, i.e., a negative inotropic effect developed. The f–F curve with positive and negative inotropic action of the low-sodium solution are illustrated in Fig. 2. As Fig. 2B shows, the f–F curve, as it rises upward along the scale of amplitude, changes from monophasic to triphasic. In the presence of a negative inotropic effect the f–F curve was triphasic and monophasic (Fig. 2C).

Complete removal of K ions from the Tyrode's solution caused a positive inotropic effect in 14 of 17 preparations, but in 3 experiments the amplitude of contractions fell. In these cases the inotropic action of the potassium-free solution coincided with the negative inotropic effect of the low-sodium medium. In nine preparations after removal of K from the Tyrode's solution the amplitude of contractions increased, whereas in the same preparations but with a decrease in the Na concentration it fell. The shape of the f–F curve in potassium-free solution as a rule was monophasic, but in some experiments it also had a triphasic appearance. The first contraction of the strip of myocardium after a long interval of rest was modified in low-sodium and potassium-free solutions in the same way as all the subsequent contractions: With a positive inotropic effect it increased and with a negative inotropic effect it decreased. The gradient of the Bowditch treppe was unchanged. A twofold increase in the external Ca concentration always led to the development of a positive inotropic effect, and under these circumstances the gradient of rise of the ladder was sometimes increased (Fig. 3).

The second group of preparations (23) had a triphasic type of f–F curve. In most experiments of this group the triphasic character of the curve was ill-defined (Fig. 2). Strength–contraction relations in Tyrode's solution with an altered concentration of Na, K, and Ca ions did not differ in principle from those which developed under similar conditions in the myocardium of warm-blooded animals [3-5, 10]. A partial decrease in the external Na concentration, the complete removal of K, and a twofold increase in the Ca concentration in the Tyrode's solution caused a positive inotropic effect in the patients' myocardium. However, there were exceptions in the preparations of this group: In two experiments a negative inotropic effect developed. This fact can be regarded as an indication of the functional similarity between the preparations of the second group, with an ill-defined triphasic type of f–F curve, and preparations of the first group with a monophasic f–F curve. In both groups of preparations alternation of the rhythm was observed, in the form of regular alternation of contractions of high and low amplitude. Alternation developed within the frequency range from 2 to 5 Hz. No connection could be found between the level of alternation and the character of the frequency–contraction relations.

The investigation thus revealed the following criteria of myocardial insufficiency: 1) a decrease in amplitude of contractions in a rhythmic series even during stimulation at frequencies of 1-3 Hz; 2) a monophasic character of the frequency–strength curve; 3) absence of positive or the appearance of a negative inotropic effect in response to a decrease in the external Na ion concentration or removal of K ions from the solution. In accordance with modern view, an increase in the amplitude of contractions during repetitive stimulation of myocardial preparations (a positive Bowditch's treppe) is caused by the fact that Ca ions passing through the excitable membrane from the external medium replenish the Ca reserves in the sarcoplasmic reticulum of the heart cells [13]. The writers showed previously in experiments on the guinea pig myocardium that compound D-600 (a verapamil derivative), which blocks the slow Na-Ca current, converted the positive Bowditch's treppe into negative during repetitive stimulation, and with an increase in the frequency of stimulation the fall in the amplitude of contractions was intensified [2]. The shape of the treppe and the f–F curve when D-600 was used were the same as in the first group of myocardial preparations tested in the present experiments, where they were interpreted as evidence of myocardial failure. It has also been shown that the partial removal of Na or total removal of K from Tyrode's solution increases the amplitude of contractions against the background of the action of D-600 in the guinea pig myocardium also. It can be postulated on the basis of these data that one cause of the appearance of the negative treppe during repetitive stimulation of preparations of the human myocardium is certain changes in the system of Ca channels (possibly a decrease in their density) caused by hypertrophy of the muscle fibers and by pathological changes in their ultrastructure [1]. The absence of a positive inotropic effect in low-sodium or potassium-free solutions may be connected with loss of the ability of the sarcoplasmic reticulum to retain Ca ions effectively during diastole and to give them up during excitation. This hypothesis is confirmed by results showing that the ability of the myocardial reticulum of patients with valvular defects to accumulate Ca ions is disturbed [7, 11], and also by the results of electron microscopic studies which show marked changes in the structure of the sarcoplasmic reticulum of the cardiac cells of these patients [1]. However, the presence of a negative inotropic effect in a low-sodium solution and a positive inotropic effect in a potassium-free solution in the same preparation show that our ideas of the nature of cardiac failure are still only very approximate.

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TISSUE RESPIRATION ENZYMES AND OXIDATIVE PHOSPHORYLATION IN THE MYOCARDIUM AFTER NEUROGENIC INJURY

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After neurogenic injury to the myocardium caused by electrical stimulation of the aortic arch for 3 h a marked increase in succinate dehydrogenase activity, a sharp decrease in cytochrome oxidase activity, and uncoupling of oxidation and phosphorylation were observed (esterification of inorganic phosphate was reduced whereas the oxygen consumption was unchanged and the P/A ratio reduced). It is concluded that in neurogenic injuries to the myocardium the coordination of regulation of energy metabolism is disturbed, with the ultimate result that oxidative phosphorylation is uncoupled and the synthesis of high-energy compounds reduced.

KEY WORDS: neurogenic injury to the myocardium; respiratory enzymes; oxidative phosphorylation.

Previous investigations in the writer's laboratory have shown that the morphological changes in the myocardium resulting from neurogenic injury caused by the action of extremal factors are preceded by metabolic disturbances. These disturbances are manifested as stimulation of glycolysis, as shown by increased hexokinase and total lactate dehydrogenase activity and by the accumulation of lactic acid [3, 4]. Meanwhile an increase in the contribution of the pentose phosphate pathway of carbohydrate oxidation to the energy metabolism of the injured myocardium is observed, as shown by increased glucose-6-phosphate dehydrogenase activity [4].

It was accordingly decided to investigate the state of oxidative processes in the myocardium following neurogenic injury. For this purpose the activity of respiratory enzymes such as succinate dehydrogenase (SD) and cytochrome oxidase (CO) and the intensity of oxidative phosphorylation were studied.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 3.0-3.5 kg. Neurogenic injury to the myocardium was produced by electrical stimulation of the aortic arch for 3 h by means of an electrode introduced through the right common carotid artery [1]. The animals were killed immediately after the end of electrical stimulation. Intact animals served as the control. Activity of SD [8] and CO [7] was determined spectrophotometrically in mitochondria isolated from the heart by differential centrifugation. The activity of the enzymes was expressed in conventional ΔE units/mg protein/h. The intensity of oxidative phosphorylation was determined manometrically in a Warburg apparatus and the protein concentration by Lowry's method [9].

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