It can be tentatively suggested that this is an influence either of electrical pulses of the stimulator or of bioelectrical currents generated by the atrium during excitation, or the mechanical activity of the atrium on the formation of the general pacemaker rhythm.

In series III (eight experiments) the effect of electrical pulses of the stimulator on the change in frequency of SV was investigated. To rule out any possible influence of the atrium, the atrium was not stimulated. Excitation of the atrium took place at the frequency assigned by SV. Stimulating electrodes were lowered into the Ringer's solution at the same place as during AS (the atria were shifted to one side so that the electrodes did not touch them). During stimulation the frequency was gradually increased, just as in the experiments of series I. Pulses of the same amplitude as in the previous series were applied to the electrode, and changes in the frequency of SV were recorded. The action of the stimulating pulses caused no change in the frequency of SV. The results of this series of experiments showed that electrical pulses of the stimulator itself do not affect the frequency of SV. The frequency of SV changes only when the atria are excited.

During high-frequency AS the sinus node thus alters its own frequency. The change in frequency of the sinus node depends on the frequency of AS. Activity of the atrium has some influence on the formation of the rhythm of SV and this influence, moreover, is exerted by some means other than retrograde conduction of excitation. Evidently under the conditions of the present experiments some influence of mechanical activity of the atrium on frequency formation in SV likewise cannot be ruled out.

Changes in rhythmic activity and the possibility of onset of arrhythmias in the sinus node of the heart must be taken into consideration in clinical practice when electrical stimulation of the atrium is carried out.

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POSITIVE INOTROPIC ACTION OF BLOOD PLASMA ON RABBIT HEART PAPILLARY MUSCLE

E. G. Vornovitskii, N. A. Len'kova, L. A. Vasilets, and B. I. Khodorov

UDC 612.172.014.46:615:38]-085.23

KEY WORDS: blood plasma; positive inotropic action; rabbit heart papillary muscle.

When studying the action of blood plasma from rabbits in a state of burn shock on electrical and contractile activity of the papillary muscles, we used blood plasma from healthy animals in control experiments [1]. When so doing we found that the addition of control plasma in Tyrode solution (in the ratio of 1:1) causes a marked increase in amplitude of the contractions of the papillary muscles. This served as the starting point for the present investigation.

Its aim was to study the effect of normal blood plasma on ionotropism–rhythm relations in the rabbit myocardium and on the resting potential (RP) and action potential (AP) of myocardial cells. To study the nature of the cardiostimulating factors in blood plasma, propranolol, a β-adrenoblocker, was used.

Laboratory of Biophysical Research, Department of Experimental and Clinical Physiology, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Ékskperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 10-13, October, 1982. Original article submitted February 28, 1982.

0007-4888/82/9410-1320$07.50

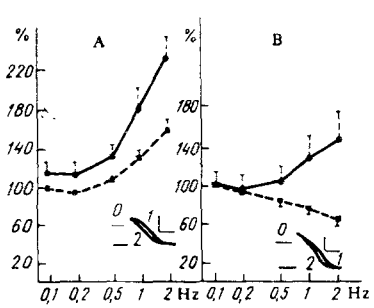

Fig. 1

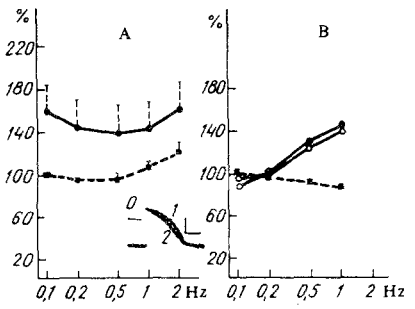

Fig. 2

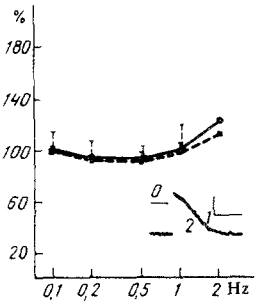

Fig. 3

Fig. 1. Increase in amplitude of isometric contractions of papillary muscles of rabbit heart and shortening of intracellular APs under the influence of blood plasma. A) Ascending type of frequency – force curve, B) descending type. APs shown in bottom part of figure: 1) in Tyrode solution, 2) in plasma. Horizontal line – isoelectric line. Squares denote Tyrode solution, circles – plasma. Abscissa, frequency of stimulation (in Hz); ordinate, amplitude of contractions (in %, amplitude of contractions during stimulation with frequency of 0.1 Hz taken as 100). Calibration: vertical 50 mV, horizontal 100 msec.

Fig. 2. Preservation of positive inotropic action of plasma on contractions of papillary muscles after addition of propranolol. Empty circles – plasma with propranolol in a dose of 1×10^{-6} g/ml. Remainder of legend as to Fig. 1.

Fig. 3. Absence of positive inotropic action of dialyzed plasma. Legend as to Fig. 1.

EXPERIMENTAL METHOD

Experiments were carried out on the papillary muscles of the right ventricle of the rabbit heart. Rabbits weighing 2-3 kg were anesthetized with urethane (1 g/kg body weight) and blood taken from the carotid artery. Thoracotomy was quickly performed and the heart removed and placed in oxygenated Tyrode solution at room temperature. Papillary muscles not more than 0.8-1 mm in diameter and 4-6 mm long were dissected. A metal ring was fixed to the tendon of the papillary muscle. The muscle was isolated at its base with a piece of endocardium. The preparation was placed in a 2-ml working chamber. The base of the muscle was attached to a hook fixed into the floor of the chamber. The apex of the muscle was attached by the ring tied to it to the rod of a 6MKh1S mechanotron. The initial load on the preparation was 1.5-2 g. This load ensured the maximal amplitude of contraction of the muscle. During the 60 min before the experiment began the papillary muscles were stimulated by square pulses 5-10 msec in duration, with a frequency of 1 Hz, and with twice the threshold amplitude. In the course of the experiment the muscle was stimulated with frequencies of 0.1, 0.2, 0.5, 1, and 2 Hz, moving successively from one frequency to another. An ÉSU-2 stimulator was used. Contractions were recorded on the N-306 automatic writer. Intracellular transmembrane potentials (RP and AP) of the papillary muscles were recorded by "floating" glass microelectrodes filled with 2.5 M KCl solution. The microelectrodes were connected by means of an adaptor to an MZ-3B cathode follower (Nihon Kohden, Japan). The output of the cathode follower was connected to the input of an S1-16 oscilloscope, from the screen of which the potentials were recorded.

The preparations were perfused initially with Tyrode solution of the following 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. The solution was saturated with carbogen (95% O_2 and 5% CO_2). The temperature of the perfusion fluid in the working chamber was kept between 35 and 36°C; pH 7.2-7.4.

After ionotropism –rhythm relations and intracellular potentials had been recorded in Tyrode solution, the preparation was perfused in blood plasma diluted 1:1 with Tyrode solution. The plasma was obtained by centrifugation of heparinized blood at 6000 rpm for 20 min. Contractions and electrical potentials were recorded 30 min after the beginning of perfusion with plasma.

Dialyzed plasma was used in a special series of experiments. Dialysis was carried out against 30 volumes of Tyrode solution at 5°C for 24 h, with two changes of solution. An artificial kidney membrane, permeable for substances with molecular weight of under 30,000-50,000 daltons was used for dialysis. Antifoam was used to prevent frothing during oxygenation of the plasma.

TABLE 1. Effect of Blood Plasma on Intracellular Potentials of Rabbit Heart Papillary Muscle (n = 15)

Solutions	RP, mV	AP, mV	Duration of AP, msec, level of repolarization		
			20%	50%	80%
Tyrode	83 ± 2,2	113 ± 5,0	61 ± 6,9	127 ± 9,1	183 ± 9,9
Tyrode + plasma (1:1)	85 ± 3,6	111 ± 5,7	58 ± 5,5	161 ± 8,2*	157 ± 7,5*

*P < 0.01 (method of matched pairs).

There were three series of experiments in which inotropism–rhythm relations and electrical potentials were studied in Tyrode solution and normal blood plasma (series I, 47 preparations), in Tyrode solution and blood plasma containing 1×10^{-6}–4×10^{-6} g/ml propranolol (series II, 11 preparations), and in Tyrode solution and dialyzed blood plasma (series III, 10 preparations).

EXPERIMENTAL RESULTS

In the experiments of series I in 31 cases the frequency – force (f–P) curve in Tyrode solution was ascending in character, i.e., the amplitude of steady-state contractions at a given frequency increased with an increase in the frequency of stimulation from 0.1 to 2 Hz. A descending type of f–P curve was found with 16 preparations: with an increase in the frequency of stimulation the amplitude of the contractions fell continuously or remained unchanged. Ascending and descending types of f–P curves in Tyrode solution of the usual ionic composition and their changes on the addition of blood plasma to this solution are illustrated in Fig. 1. In the ascending type of curve (Fig. 1A) blood plasma increased the force of contractions of the preparations at all frequencies of stimulation. The greatest increase in contraction was observed as a rule at frequencies of 1 and 2 Hz. Intracellular potentials were recorded in 15 experiments under the influence of normal blood plasma at a frequency of stimulation of 1 Hz. Values of RP and AP recorded in 28 cells in Tyrode solution and in 32 cells in normal blood plasma are given in Table 1. No significant change in RP and AP was found when perfusion with Tyrode solution was replaced by perfusion with blood plasma. The duration of APs measured at levels of 20, 50, and 80% from the peak of the AP was significantly reduced under the influence of blood plasma (Table 1; Fig. 1A).

The action of plasma on contractions of the preparations when the f–P curve was of the descending type is illustrated in Fig. 1B. Under the influence of plasma the character of inotropism–rhythm relations was found to be changed, from descending the f–P curve changed into ascending, and the amplitude of the contractions was significantly increased. Meanwhile, just as with the ascending type of f–P curve, the duration of the AP was shortened (Fig. 1B).

In 22 preparations maximal velocities of contraction and relaxation were measured in Tyrode solution and in plasma. To eliminate scatter of the initial values, velocity was normalized for the same amplitude of contractions. The normalized maximal velocity of contraction (P_{max}), measured at a frequency of stimulation of 1 Hz, was 8.03 ± 0.46 sec^{-1} in Tyrode solution and 8.98 ± 0.61 sec^{-1} in plasma. Statistical analysis of the results by the matched pairs method showed a significant increase ($P < 0.05$) of the normalized P_{max} in plasma. This means that plasma increased both the amplitude and the maximal velocity of the contractions; the increase in P_{max} was greater than the increase in amplitude. The normalized maximal velocity of relaxation was 5.07 ± 0.45 sec^{-1} in Tyrode solution and 5.77 ± 0.35 sec^{-1} in plasma. The difference was not statistically significant ($P > 0.05$), which indicates that the velocity of relaxation in most preparations changed by the same degree as the amplitude of contraction.

To discover the cause of the positive ionotropic action of plasma on the amplitude of contraction of the preparations two special series of experiments were carried out. In one of them (series II) propranolol, which blocks β-adrenoreceptors of the heart cell membrane, was added to the plasma in a dose of 1×10^{-6}–4×10^{-6} g/ml. The action of plasma containing propranolol on the inotropism–rhythm relations for preparations with ascending (Fig. 2A) and descending (Fig. 2B) types of frequency–force curve is shown in Fig. 2. With the ascending type of curves, plasma in the presence of propranolol increased the amplitude of contractions equally at all frequencies of stimulation. In four of the five experiments of this series in which APs were re-

corded, the duration of the APs was shortened (Fig. 2A). Propranolol did not prevent conversion of the descending type of curve into the ascending type (Fig. 2B). Similar results were obtained in four experiments. Propranolol thus did not abolish the positive inotropic action of plasma on the amplitude of contractions of rabbit heart papillary muscles. This evidently indicates that the action of plasma is not attributable to the presence of catecholamines, exerting their action through β-adrenoreceptors in it.

In the experiments of series III the plasma was dialyzed, whereby all substances with a molecular weight of under 50,000 daltons were removed from it. These experiments showed that dialyzed plasma did not increase the amplitude of contractions of the papillary muscles in comparison with their amplitude in Tyrode solution. It will be clear from Fig. 3 that all the points on the f–P curve almost completely coincided in Tyrode solution and in plasma. Only a small increase in the amplitude of contractions occurred with a frequency of stimulation of 2 Hz, and it was not significant. In five experiments in which intracellular potentials were recorded, the amplitude and duration of the AP were completely identical in Tyrode solution and in dialyzed plasma.

The investigation thus showed that replacement of Tyrode solution by blood plasma leads to an increase in amplitude of contractions of rabbit heart papillary muscles in response to their repetitive stimulation. The positive inotropic action of plasma is accompanied by shortening of the duration of intracellular APs. Since the cause of an increase in amplitude of the contractions could be a higher concentration of Ca^{++} ions in blood plasma compared with Tyrode solution, the free Ca^{++} ion concentration was measured in plasma and in Tyrode solution (three experiments) by means of selective calcium electrodes (the PHM64 pH-meter from Radiometer, Denmark). The concentrations of Ca^{++} ions in the plasma and Tyrode solution were equal.

The increase in amplitude of the contractions was evidently not connected with the action of catecholamines, which may be present in plasma, for propranolol, an adrenoblocker, did not abolish the positive inotropic action of plasma. These findings are in agreement with the results of an investigation [2] in which it was shown that the addition of propranolol to blood used to perfuse the dog heart papillary muscle caused no changes in the force of contractions of the muscle stimulated at different frequencies. It can be tentatively suggested that shortening of the duration of AP with an increase in the force of contractions is due to elevation of the intracellular Ca^{++} ion concentration, which leads to an increase in the outward potassium currents, which accelerates the development of the repolarization phase of the AP [3].

The absence of any positive inotropic effect of dialyzed plasma suggests that the factor which increases the force of contraction occurs in the low-molecular-weight fraction of plasma (under 50,000 daltons), which is removed by dialysis.

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