ON THE MECHANISM OF PHARMACOLOGICAL CARDIOPLEGIA

L. V. Rozenshtraukh and A. P. Fedorova

From the Department of Human and Animal Physiology (Head, Active Member of the Akad. Med. Nauk SSSR Professor A. V. Lebedinskii) of the Bio-Soil Faculty of the M. V. Lomonosov Moscow State University
(Presented by Active Member of the Akad. Med. Nauk SSSR A. V. Lebedinskii)
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Surgery on cardiac defects is closely linked with the problem of cardioplegia. At the present time, there is an increasing tendency toward the opinion that there are harmful sequelae from cardioplegia induced by pharmacological agents. Thus Björk and Fors [11], performing experiments on dogs in which they studied the changes in the myocardium which arise after stoppage of the heart by the use of potassium and acetylcholine, observed a reduction in the concentration of glycogen and the appearance of hemorrhages within the myocardial tissue. After anoxic arrest, caused by clamping the ascending aorta, they noted less manifest structural changes, but in a number of cases they observed a temporary insufficiency of the tricuspid valve. The authors considered it safest to stop the heart through chilling (to 10°), by means of perfusing the coronary vessels with cooled blood. Other authors have noted a preference for hypothermic cardioplegia too [1,4,10,14]. The data of electron microscope investigations also give evidence of morphological changes following pharmacological cardioplegia [12].

It must be noted that chilling of the myocardium, which does not cause significant morphological changes, may be traumatic functionally. This is indicated by a series of experimental data showing that cooling is one of the factors potentiating fibrillation [3]. Thus, apparently, stopping the heart under conditions of profound myocardial hypothermia cannot be considered a faultless method. At the same time, it should be recognized that the pharmacological cardioplegic agents are far from exhausted, and the mechanism of action of those agents which have been used is not yet definitively elucidated; thus, a more detailed study of the mechanisms of cardioplegia should naturally assist in demonstrating the conditions that inhibit rapid and complete restoration of cardiac activity following cardiac arrest.

Up until recently, potassium citrate was the most frequently used agent for cardioplegia in surgical practice [13]. As was shown [6], reversible cardiac arrest can be obtained under experimental conditions by the use of potassium salts of the acids participating in the Kreb's cycle (potassium malate, succinate, and fumarate). Continuing this work, we studied the effect of the K^+ cation, and the anion of the different salts used, on the mechanical and electrical activity of the myocardium. For this purpose, we used the functional unit of the myocardium – the trabecula.

EXPERIMENTAL METHOD

The experiments were carried out on isolated trabeculae from the right auricle of Rana temporaria L. Opening and unfoling of the isolated heart was accomplished according to the modified method used earlier [7,8]. The structure of the auricle was clearly visible in the open heart, consisting of coarsely and finely trabeculated (honeycombed) layers. The first layer was made up of trabeculae having predominantly a longitudinal direction, and connected with one another by very big anastomoses. The second layer contained thinner trabeculae, arranged in a honeycomb fashion. In the experiments, we used the coarse trabeculae of the right auricle (from 1 to 3), arranged in a parallel bundle. After stretching out the auricle, we removed the connective tissue layer which covers the trabecula, and then separated the trabecular bundle from its neighboring tissue with a thin, glass dissecting rake. The isolated bundle, together with a small amount of ventricular tissue, was cut with scissors, and a thin silk ligature was applied to the free end. The other end of the trabecula was directly connected to structures in the auricle. After this, the auricles were separated from the ventricle and stretched out with glass pins on a cork sheet coated with paraffin. The ligature was connected from the trabecula to a light writer, consisting of a hollow glass capillary, and the mechanical activity was recorded on a kymograph. Contraction of the auricles was not reflected on the mechanogram of the trabecula, since the auricles were tightly fixed by the glass pins. For visual observation of the trabecula's bioelectric activity, we used the type ÉNO-1 electron-ray oscillograph; the sensitivity of the apparatus was elevated by introducing a direct current

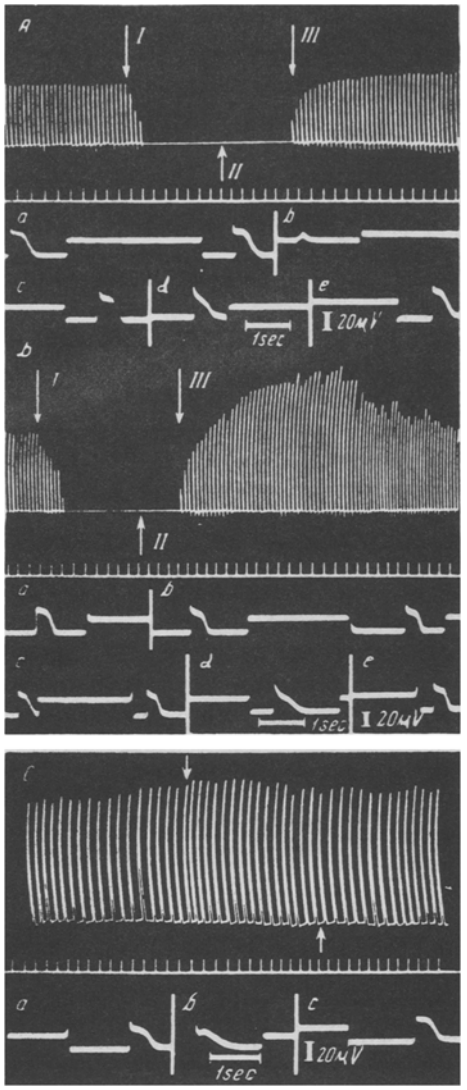

Fig. 1. The effect of potassium citrate (A), sodium citrate (B), and the solution (Citr. Na + $CaCl_2$) (C), on the electrical and mechanical activity of the trabecula; A,B) on the kymogram; |I) application of the test solution; |II) washing with Ringer's solution; |III) application of $CaCl_2$ solution; time markings (5 seconds); on the oscollogram: a) original electrogram (EG); b) EG at the moment of action of the test solution; c) EG after washing with Ringer's solution; d) EG after application of a solution of $CaCl_2$; e) EG after washing with Ringer's solution; time markings (1 sec), calibration with 20 mv; C) on the kymogram: |) application of the test solution; |) washing with Ringer's solution; time markings (5 sec); on the oscillogram: a) original EG; b) EG at the moment of action of the test solution; c) EG after washing with Ringer's solution; time markings (1 sec), calibration, 20 mv.

preamplifier into the working scheme, with a conduction band of $0\text{-}2 \cdot 10^3$ hertz. The biopotentials were conducted by DuBois-Ramon nonpolarizing electrodes with finely drawn tips, and for conduction of the resting potential and the monophasic action potential, the small amount of ventricular tissue at the distal end of the trabecula was cauterized. The indifferent electrode was placed in this area, and the conducting electrode was situated in the intact portion of the trabecula. The potentials observed on the oscillograph screen were recorded by a shleifnii oscillograph. The salts were prepared from dissolved acids (citric, malic, succinic, and fumaric) by neutralization with alkali to a pH of 7.0-7.2, in a concentration of 0.6-0.8%. Application of the salt solutions was carried out with a thin pipette onto the isolated trabecula, so that the solution did not fall on the auricle. We studied the effect on contraction and excitation of both potassium and sodium salts of these acids. The solution concentration used (0.6-0.8%) excluded a change in the ionic ration of sodium-potassium, which permitted us to show the effect of the anion of the applied salt. The duration of action of the material was limited to 40-60 sec, after which the trabecula was washed with Ringer's solution (NaCl – 6.5 g, KCl – 0.14 g, $CaCl_2$ 0.13 g, $NaHCO_3$ – 0.15 g, H_2O – 1000 ml). The electro- and mechanogram of the trabecula were recorded continuously throughout the entire experiment.

EXPERIMENTAL RESULTS

Fig. 1A shows that potassium citrate caused a drop in the membrane potential and cessation of mechanical and electrical activity. After washing of the trabecula, the membrane potential was restored and the electrogram appeared, but mechanical activity remained absent. The action of a 0.6% solution of $CaCl_2$ on the trabecula led to restoration of the mechanical activity, the amplitude of the contractions in the mechanogram exceeded the original, and the electrogram became more continuous as a result of the action of calcium. With a surplus of Ca^{++} ions, we observed the same changes in the form of the monophasis action potential as were described under the conditions of microelectrode conduction [16]. After the action of sodium citrate (Fig. 1B), we observed cessation of mechanical action in the trabecula, but retention of the electrogram. Application of calcium chloride to the trabecula restored the mechanogram (Fig. 1B). For a more complete control of the action of citric acid, we used a mixture of equimolecular amounts of sodium citrate and calcium chloride

$$2Na_3(C_6H_5O_7) + 3CaCl_2 = 6NaCl + Ca_3(C_6H_5O_7)_2 .$$

Application of this solution had no essential effect on the electrical and mechanical activity of the trabecula, although in certain cases we recorded changes in the electrogram that were typical of excessive concentrations of calcium (Fig. 1C).

The solutions of potassium malate, succinate, and fumarate (Fig. 2, I, II, III) caused a decrease in the membrane potential, and the disappearance of mechanical and electrical activity. After washing with Ringer's solution, we observed complete

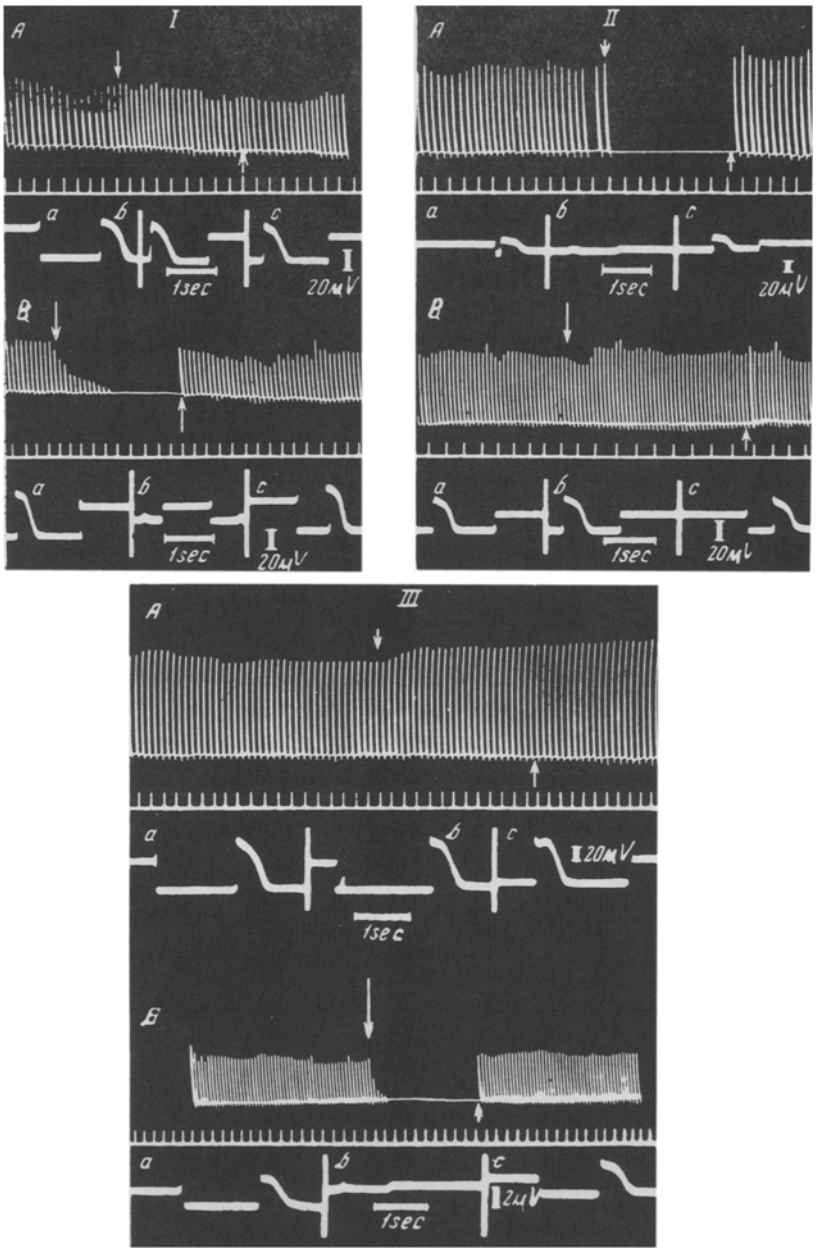

Fig. 2. The effect of sodium succinate (I A) and potassium succinate (I B), sodium malate (II B) and potassium malate (II A), sodium fumarate (III A) and potassium fumarate (III B), on the mechanical and electrical activity of the trabecula. Designations are the same as in Fig. 1 C.

restoration of both indices. Solutions of the sodium salts of these same acids did not essentially alter the mechanical and electrical activity of the trabecula, although in each case there was a certain difference in the restoration period.

Of all the tested salts of acids from the Kreb's cycle, only the citric acid anion inhibited the restoration of mechanical activity in the trabecula, despite complete normalization of the electrogram. Restoration of the mechanogram after the addition of calcium testifies to bonding of the Ca^{++} of the trabecula with the anion of citric acid. Obviously, an exchange reaction arises in this case, as a result of which the anion of citric acid combines with the Ca^{++} of the substrate, and the potassium either exists in the free state or enters into an easily broken bond with some active group of the substrate. With the action of sodium citrate on the trabecula, the sodium ion exerts no effect, since the solution is isotonic for the trabecula with relation to sodium. Nonetheless, the mechanical activity is stopped as a result of calcium blockade. The capacity of citrate to bind calcium is widely known, but in this case it has

special significance. During cardioplegia a high concentration of potassium citrate was introduced into the coronary system: 2 ml of a 20% solution in 18 ml of aerated blood [13]. Nowadays, the concentration has been reduced by a factor of ten, but there are still bases for considering 2-2.5% solutions as too high a concentration. This is evidenced by the following calculation: normal serum contains an average of 10 mg%, and erythrocytes – 3 mg% of calcium [5]; thus, in 18 ml of whole blood there are 2.34 mg of calcium. Two ml of a 20% solution of citrate contain 400 mg of calcium, and correspondingly, in a 2% solution, 40 mg.

Therefore, in using potassium citrate in the doses usually applied, the calcium reserve of the blood is markedly exceeded. The myocardial calcium citrate probably will not bind with the anion only when there is a decrease in the concentration of potassium citrate of more than 5-50 times, but with these concentrations it is difficult to expect the cardioplegic effect. It is possible that during cardioplegia there is a partial binding of the anion of citric acid with the calcium of the myocardium. As a result of this, most likely, after citrate cardioplegia one observes a decrease in myocardial tonus, the appearance of fibrillation, and other disturbances, which hamper restoration of normal activity in the myocardium after cardioplegia.

The obtained results can explain the experimental data indicating changes in the activity of the heart following transfusion with citrated blood [2,9].

In addition, the separation of electrical and mechanical activity in the trabecula, attained by the blockade of calcium, testifies that calcium ions are necessary for the effective interaction of actin, myosin, and phosphates. In connection with this, it is interesting to point out the data [15] showing that a third of all the polyphosphates present in muscle are inosinphosphates, where the calcium atom forms a chelate compound. The data which we obtained permits postulating that one of the possible reasons for the absence of mechanical activity associated with binding of the trabecular calcium is the blockade of calcium in the chelate compound, as a result of which the energy of ~P bonds cannot be transferred to the protein.

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