mals compared with the relatively small reduction in amplitude in the cats of group 2 point to the greater power of its visual commissural inputs in the telencephalon compared with the subcortical inputs. Preservation of low-amplitude EPs in the left hippocampus in the cats of group 3 was evidently connected with the conduction of visual information into this structure via interhemispheric communications in the floor of the third ventricle and the brainstem reticular formation.

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EFFECT OF PHOSPHATE AND ACIDOSIS ON SENSITIVITY OF CARDIAC MYOFIBRILS TO CALCIUM

V. I. Veksler, E. P. Elizarova, and V. I. Kapel'ko

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The force developed by muscle fibers is known to depend on the Ca^{++} concentration in the medium surrounding the myofibrils. However, various factors can modify this dependence. One such factor is the phosphate concentration. It has been shown that phosphate lowers the sensitivity of skeletal fibers with chemically destroyed sarcolemma to Ca^{++}, and also reduces the maximal developed tension [1]. An increase in phosphate concentration leads to a decrease in isometric tension of the glycerinized rabbit papillary muscle [7]. However, it is not known to what degree phosphate modifies sensitivity to Ca^{++} or maximal tension of heart muscle fibers. Acidosis is another factor which changes the sensitivity of myocardial fibers to Ca^{++} [4]. In myocardial ischemia, acidosis [3, 8] and phosphate accumulation through breakdown of creatine phosphate and ATP [2, 9], usually develop simultaneously.

The aim of this investigation was to study the effect of phosphate on calcium sensitivity of myocardial fibers with hyperpermeable sarcolemma at neutral pH and under conditions of acidosis.

EXPERIMENTAL METHOD

The method of preparation of bundles of fibers with hyperpermeable sarcolemma was basically the same as that described previously [11]. Male Wistar rats were decapitated, and the heart was removed and transferred into Krebs' solution at 0-4°C. Bundles of muscle fibers 0.2-0.3 mm in diameter were separated from the endocardial surface of the left ven-

Laboratory of Physiology of the Myocardium, Institute of Experimental Cardiology, All-Union, Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR E. I. Chazov.)

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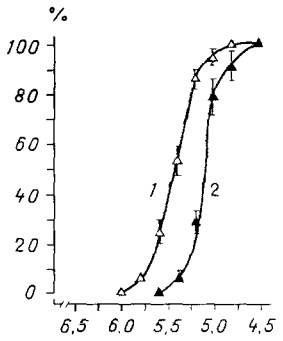

Fig. 1

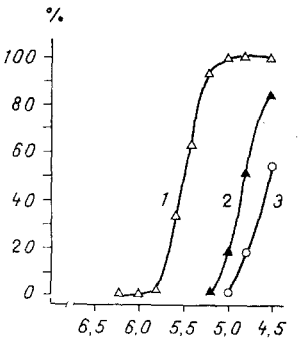

Fig. 2

Fig. 1. Relative isometric tension of bundles of myocardial fibers depending on Ca^{++} concentration in absence (curve 1, 12 experiments) and in presence (curve 2, eight experiments) of 10 mM phosphate. Abscissa, negative logarithm of pCa; ordinate, relative tension. Values of tension in absence and presence of phosphate at pCa = 4.5 taken as 100%.

Fig. 2. Relative isometric tension of bundle of myocardial fibers depending on Ca^{++} concentration at pH 7.0 and 6.6. Abscissa, pCa; ordinate, relative tension: 1) pH 7.0, without phosphate; 2) pH 6.6, without phosphate; 3) pH 6.6, 10 mM phosphate, Tension in medium without phosphate at pH 7.0 and pCa 4.5 taken as 100%.

tricle and were transferred into a solution of ethyleneglycol-bis-(β-aminoethyl ester)-N,N-tetraacetate (EGTA), in which they were incubated at 0°C for 4-8 h. After this treatment the integrity of the sarcolemma was lost and it became "hyperpermeable" [11]. Bundles of fibers prepared in this way were transferred to a glass vessel equipped with a mixer and filled with 1 ml of basic relaxing solution (see below). The ends of the bundles were secured with tungsten clips. One clip was connected to a force transducer (FT 03, "Grass"), while the other clip was fixed to a micrometer screw, so that the length of the bundle of fibers could be controlled. A bundle suspended in the relaxed state was stretched until the appearance of contraction, and then by a further 20%. Isometric tension was measured by means of an amplifier (model 13421202, "Gould") and recorder ("Linear"). The solutions were changed with a change of vessel. The experiments were conducted at room temperature (22-23°C). The EGTA solution in which the sarcolemma of the cells became hyperpermeable consisted of 100 mM potassium methanesulfonate, 2 mM $MgCl_2$, 10 mM EGTA, 5 mM Na_2-ATP, 10 mM creatine phosphate, 10 mM imidazole, and 0.5 mM dithiothreitol, pH 7.0. All the remaining solutions used contained 3 mM free Mg^{++} and 20 mM imidazole and had an ionic strength of 0.16 M, regulated by the addition of potassium methanesulfonate. The basic relaxing solution contained in addition 5 mM Mg-ATP and 15 mM creatine phosphate. Calcium was added to the solutions in the form of Ca-EGTA. The total EGTA concentration was always 10 mM. The composition of the solutions was calculated by means of a set of equations [5], using dissociation constants [6]. Solutions were made up in distilled deionized water.

EXPERIMENTAL RESULTS

In the absence of phosphate in the incubation medium, at pH 7.0 tension of the myocardial fibers usually appeared when the free Ca^{++} concentration was about 1 μm (Fig. 1). Half-maximal tension developed when the Ca^{++} concentration (pCa) was 5.4, and maximal tension at pCa = 4.8. The presence of phosphate (10 mM) in the medium sharply reduced the sensitivity of the fibers to Ca^{++} and shifted the pCa versus tension curve to the right by about 0.3 pCa unit (Fig. 1). Phosphate also reduced the maximal tension by 18 ± 3%.

Similar effects also were found with skeletal muscle [1]. It is not yet clear why they occurred. It can be suggested that phosphate does not change the affinity of troponin C for Ca^{++} but reduces the life of the actomyosin bridge [1].

In the absence of phosphate, lowering pH to 6.6 also sharply reduced the sensitivity of the cardiac muscle fibers to Ca^{++} (Fig. 2). The pCa/tension curve was shifted to the right

by about 0.6 pCa unit. Under these conditions the presence of phosphate depressed the ability of the fibers to develop force even more. It will be clear from Fig. 2 that under these circumstances tension did not develop, even when the Ca^{++} concentration was 10 μm. The same bundle of fibers at pH 7.0 and in the absence of phosphate developed maximal tension in the presence of this Ca^{++} concentration. The combined action of phosphate and acidosis was thus manifested as a sharp reduction in sensitivity of the myofibrils to Ca^{++}.

It was postulated previously that accumulation of inorganic phosphate in the ischemic myocardium is one of the main causes of the decrease in the force of contraction of heart muscle [10]. This hypothesis was later confirmed by experiments in which perfusion of the rabbit ventricular septum with a solution containing an increased phosphate concentration reduced the tension developed by the muscle [12]. However, the workers who studied this problem explained the action of phosphate purely on the grounds that this anion binds Ca^{++} and increases its sequestration in the sarcoplasmic reticulum and mitochondria, thereby reducing the free Ca^{++} concentration in the myoplasm. The results of the present experiments show that there is another very powerful mechanism of depression of myocardial contractility by phosphate, namely a decrease in sensitivity of the myofibrils to Ca^{++}. Acidosis has a similar effect on contractility. The combined action of acidosis and an increased phosphate concentration (and both these factors are present together in a zone of ischemia) very sharply reduces the ability of myocardial fibers to develop force in response to a definite Ca^{++} concentration. This mechanism is evidently a protective one for the ischemic heart cell, for it inhibits the principal energy-consuming process, namely contraction.

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