CREATINE KINASE IN EXTRACTS OF SMOOTH AND STRIPED MUSCLES AND THE EFFECT OF PAPAVERINE ON ITS ACTIVITY

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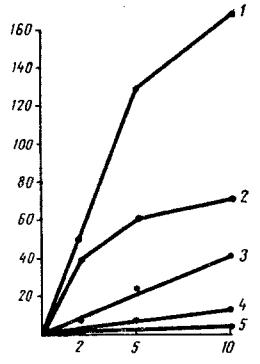
Creatine kinase and creatine phosphate (CP) play an important role in the contractile activity of striped muscles and in maintaining the tone of smooth muscle [9]. When investigating the action of papaverine on myocardial metabolism, we observed a change in the activity of creatine kinase (CK) following intravenous injection of the alkaloid into rabbits [4]. The chief pharmacological property of papaverine is its ability to lower the tone of smooth muscles, including arteries. The metabolic processes in the arterial wall have recently been studied intensively, and it has been found that enzymes of the tricarboxylic cycle [10, 14] and of glycolysis [7] are present in the aortic tissue, and that glycolysis is predominant over aerobic respiration [16]. Adenosinetriphosphatase [6, 11], myokinase [12], and various other enzymes have been found in aortic tissue. Balo and co-workers [6] could not find CK in the aortic wall, when determining this enzyme by a decrease in ATP content in the presence of creatine.

The present report describes the results of a study of the CK of smooth muscles and of the effect of papaverine on the CK of different types of muscle in different animals.



Incubation time (in min)

Fig. 1. Activity of CK in extracts of different muscles. The experiments were conducted in identical conditions in phosphate buffer at pH 7.4. Activity expressed in μmoles CP/mg protein. 1) Rabbit's skeletal muscle; 2) hen's stomach; 3) rabbit's heart; 4) dog's femoral artery; 5) rabbit's aorta.

METHOD

The heart and vessels were irrigated with physiological saline to remove the blood, and the minced muscle tissue was extracted by grinding in the cold with 0.9% NaCl solution in the proportion of 4 ml/g tissue. The extract was centrifuged at 3000 rpm and added in volumes of 0.2 ml (extract of the blood-vessel wall) or 0.05 ml (extract of heart tissue, or skeletal muscle tissue of rabbits, and of smooth muscle from the hen's stomach) per ml of sample. The incubated samples contained per ml: 7 μmole sodium salt of ATP, 16 μmole creatine, 1 μmole $MgSO_4$, 14 μmole phosphate or Tris buffer (trihydroxymethylaminomethane and HCl) and 110 μmole NaCl. Incubation was carried out at 26° and the pH of the incubation medium was 7.4. The reaction of CP formation was stopped by the addition of an equal volume of 1.4% ammonium molybdate in 2N H_2SO_4 [1]. If at the same time as the CP was determined, the concentration of inorganic phosphate was estimated, the reaction was carried out in Tris buffer. The proteins were precipitated with 5% trichloroacetic acid. Ammonium molybdate was added to the trichloroacetic extract and the amount of creatine formed was determined after 40 min with alkaline picrate (0.2 ml of test solution, 0.6 ml of 5% NaOH solution, and 1.2 ml of a saturated solution of picric acid [1, 8]). The concentrations of inorganic phosphate and protein were determined by Lowry's method [17, 18]. Papaverine hydrochloride was added to the samples in 0.1 ml of aqueous solution.

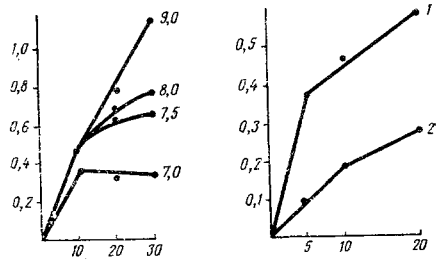


Fig. 2. Formation of CP by tissue of rabbit's aorta in relation to pH (A) and nature of buffer used (B). 1) Phosphate buffer; 2) Tris buffer. CP content expressed as μmoles per sample.

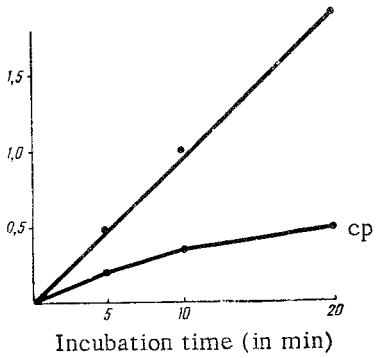


Fig. 3. Relationship between ATPase activity (in the presence of Mg) and CK activity in extract of rabbit's aorta at pH 7.4. Concentration of CP expressed in μmoles per sample, and of inorganic phosphate – in μatoms per sample.

TABLE 1. Effect of Papaverine on Creatine Kinase of Extract of Rabbit's Aorta

Concentration of papaverine, $\times 10^{-7}$ M	Amount of CP formed in sample in 10 min (in μmoles)							
	Phosphate buffer					Tris buffer		
0	0.40	0.34	0.45	0.22	0.49	0.27	0.26	0.25
1	0.59	0.41	0.95	–	–	0.27	0.30	–
2	–	0.50	0.95	0.22	0.59	0.27	0.27	–
5	–	0.60	0.59	0.36	0.57	0.27	0.26	0.29
10	–	–	–	0.24	0.54	0.32	0.26	0.23

TABLE 2. Effect of Papaverine on Adenosinetriphosphatase Activity of Extract of Rabbit's Aorta

Concentration of papaverine, $\times 10^{-6}$ M	Amount of inorganic phosphate per sample (in μmoles)				
	After incubation for 5 min	After incubation for 10 min	After incubation for 20 min		
0	0.38	0.60	1.16	1.40	1.41
0.5	0.58	1.13	2.13	1.54	1.60
1	0.58	0.80	1.60	1.61	1.73
2	–	0.80	1.60	1.89	1.73

RESULTS

CK of Smooth Muscles

During incubation of the extract from the arterial wall (femoral artery of a dog and aorta of a rabbit) with ATP and creatine CP was formed. The intensity of this process was much lower than in the case of the other muscles (Fig. 1). It is clear from Fig. 2A, which shows the relationship between CP formation and pH, that CP formation was maximal in the extract of rabbit's aorta at pH 9. At pH 7 the reaction quickly reached equilibrium, which is very characteristic of CK. The CK activity of the aorta is dependent on the nature of the buffer: in a phosphate buffer it was higher than in Tris buffer (Fig. 2B).

According to reports in the literature aortic tissue contains active ATPase, soluble in 0.9% NaCl solution, but little work has been done to study its properties. In our experiments considerable formation of inorganic phosphate was also observed from added ATP in extract of rabbit's aorta. Splitting of ATP was found to be activated by the addition of 1 μmole Mg^{++} by 100%, and if the same quantity of Ca^{++} was added – by 200%. It is clear from Fig. 3 that the ATP in the extract of the wall of the rabbit's aorta was subjected mainly to hydrolysis, and that a smaller proportion of the high-energy phosphate was transferred to creatine.

The addition of papaverine to the sample in a wide range of concentrations led to an increase in the CP formation if the reaction was carried out in phosphate buffer; in similar conditions in Tris buffer no such increase took place (Table 1). The formation of inorganic phosphate in the presence of papaverine was also increased (Table 2). It should be noted that the action of papaverine on the two processes was independent of its concentration.

TABLE 3. Activity of CK of Extract of Rabbit's Heart Muscle (in μmoles CP per sample) Depending on Buffer

Incubation time, min	Phosphate buffer	Tris buffer
1	0.19	0.16
2	0.38	0.24
5	0.70	0.51
10	1.11	0.84

The effect of papaverine on CK from another smooth muscle (the hen's stomach) was also studied. In this case the activity was much higher than in the arterial wall (see Fig. 1), but the action of papaverine was relatively weak.

CK of Extract from Rabbit's Heart Muscle

The activity of the CK of heart tissue in phosphate buffer was higher than in Tris buffer (Table 3). According to previous findings [4], papaverine in a concentration of $1 \times 10^{-7} - 1 \times 10^{-6}$ M increased CP formation during incubation in phosphate buffer; in Tris buffer no such increase was observed.

Hence, the phosphate ion is an essential condition for the manifestation of the activating effect of papaverine on CK. This distinguishing feature of the action of papaverine, together with the absence of any relationship between the concentration of the alkaloid and the degree of its effect, demonstrate that it has no direct activating action on the enzyme.

The problem of the biochemical mechanism of the action of vasodilator substances has received very little study. According to the results which have been obtained [2, 3], the tone of smooth muscles on which papaverine acts depends on the presence of specific proteins within these muscles. It is difficult to imagine, however, that the rapid decrease in tone taking place during the action of vasodilator substances was related to a change in the protein content, as has been demonstrated, for example, in the case of a chronic disturbance of vascular tone [5]. Some workers attribute the vasodilator action of pharmacological agents to their influence on energy metabolism in the vascular wall [15, 19, 20]. The muscular layer of the vessel wall is distinguished by a very low content of high-energy phosphorus compounds [13, 20]. It may be postulated on the basis of the results described in this paper that administration of papaverine leads to a decrease in the ATP content.

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