THE EFFECT OF PAPAVERINE AND IODINE ACETATE ON MYOCARDIAL CREATINKINASE

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We have shown earlier that the injection of papaverine into rabbits brought about a considerable increase in the activity of the creatinkinase of cardiac muscle [3]. This effect was observed when investigating the creatinkinase of cardiac muscle extracts in phosphate buffer (with the addition of magnesium sulfate) or when making use of homogenates to which monoiodoacetate (MIA) had been added for preventing glycolysis. After the execution of this work detailed investigations on the inhibitors of the creatinkinase of skeletal muscle were found in the literature. It appeared that sulfates, and to a lesser degree phosphates [8] and also MIA [6, 9], were competitive inhibitors of creatinkinase with respect to the complex Mg—ATP. These data from the literature and also the results of our own research work which showed that papaverine in vitro, in the absence of the inhibitors mentioned, does not increase the activity of creatinkinase [4], led us to suppose that papaverine does not activate creatinkinase directly, but merely prevents the action of inhibitors on the enzyme.

The present work is devoted to ascertaining the reliability of this hypothesis in relation to one of the inhibitors of creatinkinase—MIA.

METHODS

An extract of cardiac muscle from the rabbit, obtained by extracting the crushed tissue with 0.9% sodium chloride solution in the cold (four volumes of physiological solution to one liter of tissue), was centrifuged and kept in a refrigerator for two days in order to inactivate the adenosine triphosphatase which would have hindered the determination of the creatinkinase. Control determinations showed that the activity of the creatinkinase was not reduced after the extract had stood for two days. For determining the activity of the creatinkinase 0.1 ml of extract was incubated at 30° with the sodium salt of ATP (8 μM), creatine (16 μM) and magnesium chloride (2 μM per 1 ml of sample). The ATP was checked by chromatography and found to contain an insignificant amount of ADP. A solution of MIA, containing no free iodine, was added to each sample. The reagents were neutralized with NaOH to pH 7.4 which was maintained by 15 mM of phosphate buffer. After incubation for one, two or five min, the reaction in which phosphocreatine was formed was arrested by adding a solution of ammonium molybdate [1], having precipitated the albumin and decomposed the phosphocreatine with the formation of creatine. The method is described in more detail in a preceding work [4].

In the experiments with papaverine, the alkaloid was injected into rabbits intravenously in doses of 1.5 μg/kg on the weight of the animal, producing a dilation of the coronary vessels [2] and a number of changes in the carbohydrate-phosphate metabolism of the myocardium [3]. The heart was excised five minutes after the injection and the extract prepared. Statistical treatment of the results was carried out by Student's method [5].

RESULTS

The activity of creatinkinase was determined in the "aged" extract from the cardiac muscle of the rabbits

TABLE 1. Effect of MIA on the Activity of Creatinkinase in "Aged" Extracts from Cardiac Muscle Tissue (incubated for two minutes)

Concentration of MIA	Formation of phosphocreatine (in μg creatinine per sample					Depression of activity of enzyme, expressed as MIA, %		
	control		experiment (papaverine)			control	experiment (papaverine)	P
	number of animals	phosphocreatine	number of animals	phosphocreatine	P			
0	10	20,9	8	21,4				
0,001M	9	16,6	9	20,5	0,05	20,5	4,0	0,001
0,002M	5	13,8	6	16,7	0,05	34,0	22,0	0,01

injected with papaverine and in extracts from control animals. The activity of the enzyme was studied in three variants without inhibitor, MIA being added to the samples at concentrations of 0.001 and 0.002 M. (Table 1).

As is seen from Table 1, the activity of creatinkinase in the control rabbits and in those injected with papaverine were similar, but a difference was observed in the sensitivity of the enzyme to monoiodoacetic acid. A comparatively small concentration of MIA (0.001 M) caused a distinct depression in the formation of phosphocreatine in the control rabbits. In animals which had been injected with papaverine the difference between the samples with and without the addition of 0.001 M iodoacetate was statistically insignificant; in many experiments the addition of MIA did not affect the activity of the enzyme. On adding a large amount of MIA (0.002 M) to the samples the depressing action proved to be stronger in the control animals. This difference was transitory—it was observed after one and two minutes incubation but after five minutes the depressing action of MIA was similar in the experimental and control animals.

On account of the individual fluctuations in the activity of the enzyme and in the degree of activity of MIA, the reliability of the difference between the activity of the enzyme in the presence of MIA in the control rabbits and that in the rabbits treated with papaverine was small. However, a comparison of the depression caused by monoiodoacetate, expressed as a percentage and excluding individual fluctuations in activity, gave a difference which was highly significant (see Table 1).

Thus, the experiments showed that, in cardiac muscle extracts from rabbits injected with papaverine, the effect of the alkaloid on creatinkinase, expressed as a protective action against MIA, was preserved after the extract had been kept for two days.

A knowledge of the mechanism of the action of iodoacetate on the creatinkinase of cardiac muscle would help in understanding the nature of the effect of papaverine on this enzyme. There are articles in the literature on the mechanism of the action of MIA on purified creatinkinase from the skeletal muscle of the rabbit. It has been shown that MIA acylates the SH-group of the cysteine of the active center of this albumin. Evidently, this same group, with which the complex of magnesium with nucleotides such as Mg—ATP and Mg—ADP is incorporated, prevents the annexation of MIA [6]. Other authors have referred to the competitive relationships of the creatinkinase reaction and MIA [9].

A characteristic feature of the action of MIA is its dependence on the ionic strength of the solution [8]. The creatinkinase of cardiac muscle has not been obtained in the pure form and has been considerably less studied than the enzyme from skeletal muscle. It may be surmised (with great improbability) that the action of MIA on the creatinkinase of cardiac muscle was the same as on the enzyme of skeletal muscle. On the other hand, the results of experiments made during the preparatory work with both enzymes convince us that they differ in a number of properties, for instance, in thermostability and in their behavior in organic solvents in the presence of magnesium salts. As was mentioned above, it is necessary to carry out a more detailed study of the action of MIA on creatinkinase of cardiac muscle. We examined the effect of the reaction substrates and the ionic strength of the solution on the inactivation of creatinkinase by iodoacetate. The experiment was carried out in the following way. The extract was preincubated for two minutes at 30° with buffer or MIA in the presence of one or other of the substrates of the reaction (Table 2). During preincubation, either ATP with magnesium, or creatine with magnesium, or only magnesium were present in the samples. After the two minute preincubation the ingredients were mixed, the time being insufficient for the course of the reaction forming phosphocreatine (depending on the experimental variant).

TABLE 2. Effect of the Reaction Substrates and the Ionic Strength of the Solution on the Depression by MIA of Creatinkinase Extract of Cardiac Muscle (average data)

Composition of sample at preincubation	Substance added for starting the reaction	Concentration of MIA					
		0,001 M		0,0005 M		0,00025 M	
		mk[1]	depression, expressed as MIA, %	mk[1]	depression, expressed as MIA, %	mk[1]	depression, expressed as MIA, %
Buffer	ATP, Cr, Mg	0,155	45	0,067	42	0,067	22
Buffer, Mg	ATP, Cr	0,163	41	—	—	—	—
Buffer, ATP, Mg	Cr	0,169	29	0,081	0	0,081	0
Buffer, Cr, Mg	ATP	0,163	44	0,075	43	0,075	30
Buffer, Mg Buffer, ATP, Cr	—	0,169	7	0,081	0	—	—

*The high ionic strength (mk) was created by NaCl; in the calculations we allowed for the $MgCl_2$, phosphate and sodium added for neutralizing the ATP.

Preincubation was not possible in the sample to which creatine, magnesium and ATP were added at the same time; in this instance, MIA acted only during the time of incubation. After incubation for two minutes, the reaction was discontinued by adding a solution of ammonium molybdate and the amount of phosphocreatine formed was determined.

As is shown in Table 2, the simultaneous presence of ATP, creatine and magnesium protected the enzyme from the depressing action of MIA (lower line of table) while magnesium alone, or magnesium with creatine, was ineffective. ATP in the presence of magnesium ions exhibited a certain protective action against 0.001 M MIA and prevented the depressing action of a smaller concentration of inhibitor. In samples lacking ATP, the depression caused by MIA depended on the ionic concentration of the solution; halving the concentration increased the depression twofold (halving the concentration of MIA produced the same effect).

The results of the given experiments showed that the idea about the mechanism of the action of MIA on the creatinkinase of skeletal muscle (acylation of the SH-group of the active center to which, evidently, ATP is attached) may be extended to the enzyme of cardiac muscle tissue since, in this instance, the depression dependent on MIA is prevented by one of the substrate reactions—the complex Mg—ATP. Consequently, injected papaverine protects the creatinkinase of the myocardium from the depressing action of MIA merely by protecting the SH-group of the active center of the enzyme.

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