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Inhibition of pyruvate kinase by free fatty acids in rat heart muscle

The major substances utilized as energy sources in myocardium are carbohydrates and fatty acids. It is known that a reciprocal relationship exists between consumption of the two oxidizable substrates. While fatty acids are being utilized, glycolysis seems to be inhibited. SHIPP, OPIE AND CHALLONER[1] and OPIE, EVANS AND SHIPP[2] have demonstrated that glucose oxidation in isolated perfused rat heart decreased on addition of palmitic acid to the medium. In experiments using rat liver, WEBER AND CONVERY[3] have shown that free fatty acids produced a direct inhibitory effect on pyruvate kinase which is regarded as a rate-limiting enzyme in the glycolytic pathway. The present study was undertaken to investigate the effect of free fatty acids on this enzyme prepared from heart muscle.

Male Wistar rats weighing 150–200 g were used. They were killed by decapitation and the hearts were homogenized in 19 vol. (w/v) of ice-cold 0.25 M sucrose. The supernatant was obtained by centrifuging the homogenates for 30 min at 105 000 × *g* at 0°. Pyruvate kinase was assayed according to the method described by BÜCHER AND PFLEIDERER[4], using 55.3 mM potassium phosphate (pH 7.4) (ref. 5), 1.5 mM phosphoenolpyruvate (PEP) and 0.33 mM ADP. Fatty acids used were capric acid, myristic acid, palmitic acid and oleic acid. After being dissolved in distilled

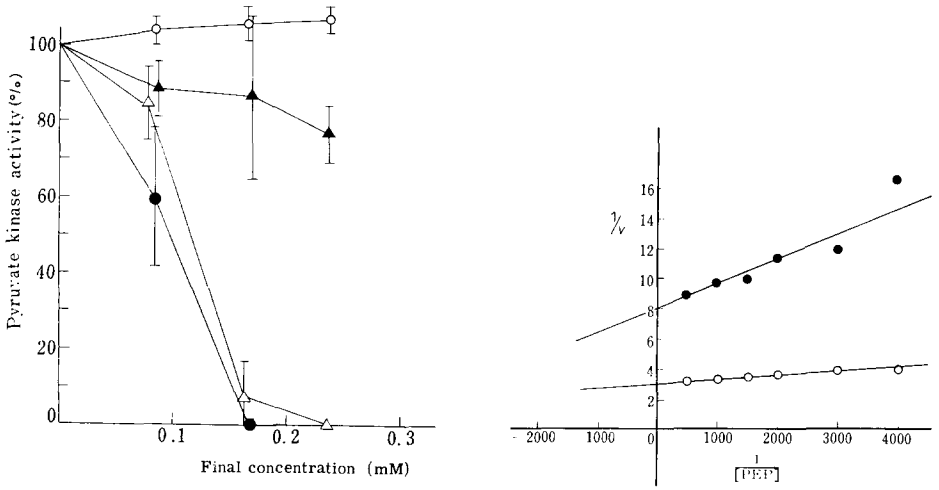

Fig. 1. Effects of fatty acid on the activity of pyruvate kinase in rat heart muscle. 0.2 ml heart supernatant was preincubated at 37° for 5 min with 0.2 ml of sodium salt of fatty acid, and a 0.2-ml aliquot from this mixture was added to reaction mixture. Control activity, taken as 100%, was measured in the supernatant without fatty acid. The concentration of fatty acids during preincubation was 2.5–7.12 mM. ○, caproate; ▲, myristicate; △, palmitate; ●, oleate.

Fig. 2. Plot of the reciprocal of initial velocity against the reciprocal of the concentration of PEP in the absence and in the presence of oleate. ○—○, without inhibitor, K_m $9.09 \cdot 10^{-5}$ M; ●—●, with 0.06 mM oleate, K_m $2.0 \cdot 10^{-4}$ M.

Abbreviation: PEP, phosphoenolpyruvate.

water, each fatty acid, at different concentrations, was first incubated for 5 min with the supernatants at 37°. At the end of this preincubation period, the mixture of supernatant and fatty acid was added to the enzyme assay reaction mixture, and the activity was determined. Supernatant protein was determined by the biuret method[6].

As shown in Fig. 1 the activity of pyruvate kinase was inhibited by palmitic acid and oleic acid in proportion to the concentration employed. Among the four fatty acids tested, oleic acid showed the greatest inhibitory action; in contrast, there was no inhibition by capric acid. The activity of the enzyme was almost completely depressed at concentrations above 0.2 mM which are attainable under physiological conditions. The levels of K_m for the substrate, PEP, were determined in the absence and in the presence of oleate. The results indicate that this inhibition is a noncompetitive one (Fig. 2). When fatty acid was added directly to the reaction mixture, there was no inhibitory effect on the enzyme activity. As shown in Table I, a more potent inhibition was noted with longer periods of preincubation. The fact suggests

TABLE I

EFFECT OF PREINCUBATION TIME ON RAT HEART PYRUVATE KINASE
The final concentration of oleate was 0.06 mM.

Preincubation time (min)	*Pyruvate kinase activity*	
	μmoles/g supernatant protein per min	*%*
0	180	100
3	129	72
5	111	62
15	59	33
30	21	12
60	0	0

that some interactions between fatty acids and the enzyme are needed before the enzyme reacts other reaction components, especially with ADP and PEP. It is possible that during the preincubation, fatty acyl-CoA is produced, which in turn manifests an inhibitory effect on the pyruvate kinase. Experiments are in progress to elucidate this point.

Palmitic acid and oleic acid are known to be utilized mainly in heart muscle[7]. A reduction of pyruvate kinase activity occurring during the increase of the intracellular concentrations of these fatty acids consequently leads to the overall inhibition of glycolysis. The biological significance of these findings may be that they indicate a regulatory mechanism involved in substrate economy of myocardial energy production.

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Sur une nouvelle forme d'ATP:arginine phosphotransférase, de poids moléculaire 160 000

A l'occasion de travaux récents, il a été établi que certaines ATP:guanidine phosphotransférases (EC classe 2.7.3), la créatine kinase (EC 2.7.3.2) et l'arginine kinase (EC 2.7.3.3), peuvent exister dans la nature à l'état actif non seulement sous plusieurs formes moléculaires (isoenzymes) mais sous plusieurs tailles moléculaires. Ainsi, la créatine kinase, dont le poids moléculaire est d'environ 80 000 chez les vertébrés[1,2] et chez certains oursins[2,3], peut atteindre un poids moléculaire double dans le muscle d'une espèce particulière, *Echinus esculentus*, et dépasser 200 000 dans les spermatozoïdes de cet animal[2]. De même, des arginine kinases de poids moléculaire environ 80 000 ont été mises en évidence dans les muscles d'oursin, de Travisia, de siponcle[3], de nombreux échinodermes[2] et de quelques mollusques[4], alors que l'arginine kinase du muscle d'écrevisse[5], de homard[6] et de nombreux mollusques[4] a un poids moléculaire d'approximativement 40 000. Ces deux types d'arginine kinase, de poids moléculaire simple et double, peuvent coexister dans certains muscles de mollusques[4].

Nous décrivons ici, à partir du muscle de deux annélides polychètes marines, *Sabella pavonina* et *Spirographis spallanzanii*, la caractérisation d'un troisième type d'arginine kinase, dont le poids moléculaire est sensiblement double de celui de l'arginine kinase d'oursin, de siponcle et de Travisia[3] et quadruple de celui de l'enzyme des crustacés[5,6]. Une réactivité particulièrement large vis-à-vis de divers analogues de la L-arginine ayant été signalée pour l'arginine kinase de Sabella[7], nous avons également entrepris d'étudier quelques caractères de spécificité de ces nouveaux enzymes.

Le travail a été exécuté sur des extraits enzymatiques acellulaires bruts de la paroi musculaire des deux vers: les tissus étaient homogénéisés en présence de deux volumes de tampon Tris–HCl 0.05 M (pH 7.5) additionné d'EDTA 0.4 mM et de dithiothréitol 0.4 mM, à 0°, et l'homogénat était centrifugé (10 min à 34 800 × *g*). Pour l'étude des spécificités, les extraits enzymatiques ont été préalablement fractionnés sur colonnes de Sephadex G-200. Les mesures d'activité ATP:guanidine phosphotransférasique vis-à-vis de la L-arginine et des divers substrats guanidiques essayés ont été effectués selon PRADEL *et al.*[8].

Le poids moléculaire de l'arginine kinase de Sabella et de celle de Spirographe a été comparé à celui de protéines standard par deux méthodes différentes:

(1) Centrifugation en gradient de densité de sucrose selon MARTIN ET AMES[9],