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ADAPTABILITY OF PYRUVATE KINASE L IN RAT LIVER, KIDNEY, AND RETICULOCYTES WITHOUT ACCOMPANYING CHANGES IN THE A ISOENZYME

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SUMMARY

1. Pyruvate kinase L has been found to be adaptive in rat kidney and reticulocytes, as previously observed for liver, increasing two-fold in response to high fructose or glycerol diets.

2. The other allosteric pyruvate kinase of liver and kidney seems to be constitutive.

INTRODUCTION

Two classes of pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.10) have been described in kidney[1]; one which accounts for about a quarter of the total activity of the crude extract and has kinetic similarities with the L class of the liver; the other, at first identified as M class, has regulatory properties similar to those described for the adipose tissue enzyme[2] and described as class A by Carbonell *et al.*[3]. On the other hand pyruvate kinase from red blood cells has been identified as L class[4]. We report here that pyruvate kinase L is an adaptive enzyme in the three tissues examined (liver, kidney, reticulocytes) while the A class isoenzyme in liver and kidney is constitutive.

MATERIAL AND METHODS

Male albino rats weighing 140–180 g were fed for seven days on a diet containing 60% carbohydrate (glucose or fructose) or glycerol, 20% caseine, 5% corn oil, 5% cellulose, 5% mineral salts and 6% vitamin mixture. Pregnant rats were fed a high fructose diet during the last week of pregnancy. In both cases control animals were fed a laboratory standard diet.

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Adult animals were decapitated and their livers and kidneys quickly removed. Tissue homogenates were prepared in 3 vol. of cold 0.25 M sucrose and the cytoplasmic fractions were obtained by centrifugation at 20 000 × *g* for 1 h, at 0–4 °C. The extracts were fractionated using 0–50% or 50–75% saturated $(NH_4)_2SO_4$ and the pellets resuspended in 25 mM Tris–HCl, pH 7.0, containing 0.1 mM EDTA and 1 mM dithioerythritol. The 0–50% fraction contains the L class pyruvate kinase while the 50–75% fraction contains the A class, as shown by Carbonell *et al.*[3].

Red blood cells were obtained from decapitated newborn rats as described by Okuda *et al.*[5], taking advantage of the fact that in newborn rats 99% of red blood cells are reticulocytes.

Assay of pyruvate kinase activity was carried out as described by Llorente *et al.*[6]. Protein determination was carried out as described by the method of Lowry *et al.*[7].

RESULTS AND DISCUSSION

Table I gives the initial and final body weights, and the liver and kidney weights. They are included since there were marked changes in several conditions. The loss of body weight of the animals on the high glycerol diet is striking, but presumably is a consequence of the poor intake of this diet. In relation to this, attempts to increase the caloric intake, reinforcing this diet by substitution of 50% glycerol for the drinking water, were unsuccessful and led to even greater loss of body weight, presumably due to dehydration. As can be seen, liver weights suffered parallel changes to body weights while kidney weights remained unaltered.

TABLE I

WEIGHTS OF THE ANIMALS AND THEIR LIVERS AND KIDNEYS

The data (presented with S.E. values) represent the means of four animals per group.

	Body weight		*Liver weight*	*Kidney weight*
	Initial	*Final*		
Standard diet	139 ± 25	162 ± 29	7 ± 0.8	1.49 ± 0.3
Starvation (48 h)	146.5 ± 24	116.5 ± 24	3.9 ± 1	1.15 ± 0.1
High glucose diet	150 ± 7	166.6 ± 7	8.5 ± 0.8	1.41 ± 0.1
High fructose diet	159 ± 8	163 ± 2	9.7 ± 1.7	1.64 ± 0.1
High glycerol diet	161.5 ± 24	110 ± 19	5.9 ± 1.9	1.38 ± 0.5

The results of an experiment in which starvation, high glucose, high fructose, and high glycerol diets were tested in their individual capacity to modify the levels of pyruvate kinase are shown in Table II. As previously reported, total liver pyruvate kinase activity decreased on starvation and increased with the high fructose diet, due exclusively to an increase in the levels of the L class. We also found a fructose-like effect for the high glycerol diet. In kidney the increase of total pyruvate kinase in response to high fructose or glycerol diets is not so significant as in liver; nevertheless, when classes L and A were estimated separately after ammonium sulphate fractionation, a two-fold increase became apparent for the L class. The difference is

TABLE II

ACTIVITIES OF PYRUVATE KINASE AND ITS ISOENZYMES IN LIVER AND KIDNEY IN DIFFERENT DIETARY CONDITIONS

Pyruvate kinase isoenzymes were separated and assayed as described in Materials and Methods. Results are expressed: A, in international units (μmoles/min) per g tissue; B, in total units (presented with S.E. values) representing the mean of four animals per group.

		Liver			*Kidney*		
		Crude extract	*Fraction L*	*Fraction A*	*Crude extract*	*Fraction L*	*Fraction A*
Standard diet	A	65 ± 3	48 ± 9	8 ± 1	47 ± 6	10 ± 1	34 ± 4
	B	455 ± 51	337 ± 80	57 ± 7	70 ± 6	15 ± 3	51 ± 6
Starvation (48 h)	A	26 ± 6*	17 ± 2*	7 ± 1	49 ± 3	11 ± 2	30 ± 3
	B	103 ± 36*	70 ± 22*	29 ± 7	56 ± 18	14 ± 4	36 ± 7
High glucose diet	A	63 ± 9	46 ± 12	6.5 ± 1	52 ± 7	10 ± 3	35 ± 6
	B	533 ± 82	386 ± 99	55 ± 9	75 ± 11	15 ± 5	50 ± 7
High fructose diet	A	110 ± 16*	72 ± 5*	7 ± 2	64 ± 5*	25 ± 2*	31 ± 4
	B	1094 ± 341*	700 ± 148*	68 ± 18	98 ± 4*	39 ± 3*	45 ± 4
High glycerol diet	A	100 ± 6*	97 ± 16*	8 ± 2	75 ± 3*	29 ± 2*	40 ± 4
	B	700 ± 189**	580 ± 186**	49 ± 16	106 ± 23*	46 ± 12*	56 ± 14

* Significant change to the control value ($P < 0.001$).
** Significant change to the control value ($P < 0.01$).

probably due to the lower level of the adaptive L class in this tissue against the predominant constitutive A class. The "high glucose" (60%) diet had no effect on the pyruvate kinases of either liver or kidney compared to the standard diet. Starvation does not affect the L class in kidney. In no case did the A class change significantly, and thus it may be considered as constitutive. Relative specific activities were consistently similar on comparing them to tissue weights as listed in the Table.

We also tested the possible adaptability of the L pyruvate kinase in red blood cells[4] to high fructose. Newborn rats having 99% of their red blood cells as reticulocytes and corresponding to pregnant rats that had received high fructose (60% and standard diets during the last week of their pregnancy) were used as the source of reticulocytes and assayed for pyruvate kinase as described in Materials and Methods. The results for the standard diet (13 newborns) and the high fructose diet (11 newborns) were 2.7 and 4.9 μmoles/min per 100 mg protein, respectively. The increase in the high fructose group was 80%, and the difference was highly significant ($P < 0.001$).

The fact that the L class of pyruvate kinase in red blood cells suffers only adaptive changes in the nucleated, immature reticulocyte cells but not in the unnucleated mature cells, which might suggest, indirectly, that the changes in the activity of the enzyme are due to its *de novo* synthesis.

Although the allosteric properties of the L class of pyruvate kinase[6] seem adequate for the fine control of this enzyme in the switch-over from glycolysis to gluconeogenesis, there is no doubt that a long term regulation involving adaptive changes in the level of the enzyme could help to minimize a futile cycle in gluconeogenic conditions while allowing for high levels when a maximal metabolic flux is expected to take place, as on the feeding of high fructose or glycerol diets. If this were the case, the above reported adaptability of the pyruvate kinase class L would

make sense in glycolytic-gluconeogenic tissues as is the case for liver and kidney, but it would not appear clear in the case of exclusively glycolytic cells as in reticulocytes. Nevertheless, considering a common ancestor for liver and reticulocyte cells (*N.B.* the hematopoietic faculty of the liver during the fetal life) the adaptability of the reticulocyte pyruvate kinase could depend on this ontogenic relationship without actual physiological significance.

In summary, pyruvate kinase L is an adaptive enzyme in the three tissues examined (liver, kidney, and reticulocytes) while the A class isoenzyme in liver and kidney is constitutive.

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