THE POSTNATAL DEVELOPMENT OF GLUCONEOGENIC ENZYMES IN GUINEA-PIG KIDNEY CORTEX

B. H. ROBINSON

Departments of Biochemistry and Paediatrics, University of Toronto, and Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada

Received 6 April 1976

1. Introduction

The capacity for gluconeogenesis in the mammalian kidney has been assumed to be less than that in liver [1] and in man assumes importance only in prolonged starvation [2]. On the other hand, the observations of Alleyne [3] and Goodman [4] have suggested that the rise in gluconeogenic capacity seen in animals with metabolic acidosis is due to the fact that gluconeogenesis and the ability of the kidney to secrete H^+ are somehow enhanced. The postnatal development of gluconeogenic enzymes in the kidney has been only partially investigated. Thus, the activities of glucose 6-phosphatase (EC 3.1.3.9.) and phosphoenolpyruvate carboxykinase (EC 4.1.1.36.) in the newborn rat kidney have been shown to increase quite substantially in the period following birth [5]. On the other hand, no detailed study of the development of gluconeogenic enzymes in the liver of animals with a bimodal distribution of phosphoenolpyruvate carboxykinase such as the guinea-pig have been reported until recently [6]. We report in this communication the appearance of key gluconeogenic enzymes in guinea-pig kidney in the period following birth.

2. Methods and materials

2.1. *Chemicals*

Special chemicals, intermediates and coenzymes were obtained from the Sigma Chemical Co., St. Louis, Missouri. Enzymes were purchases from Boehringer-Mannheim Corp., Quebec City.

2.2. *Animals*

Pregnant guinea-pigs were purchased from High Oak Ranch Ltd., Toronto, as timed pregnancies at 60 days gestation. Birth was allowed to proceed naturally at term (64-68 days) and the newborn animals remained with the mother until time of sacrifice.

2.3. *Enzyme assay in tissue specimens*

Samples of guinea-pig kidney cortex taken at sacrifice were weighed and homogenised in 10 volumes of an ice-cold buffer containing 0.25 M sucrose, 5 mM Tris HCl, and 0.1 mM EGTA, pH 7.4. The resulting homogenate was centrifuged at 600 *g* for 10 min to remove cell debris and the supernatant then centrifuged at 12 000 *g* for 10 min to give fractions containing the mitochondrial pellet and the microsomes plus the soluble components of the cells. The activity of glucose 6-phosphatase was measured by the method of Swanson [7], fructose 1,6-diphosphatase by the method of Pontremoli [8], pyruvate carboxylase by the method of Crabtree et al. [9], and phosphoenolpyruvate carboxykinase by the method of Roobol and Alleyne [10]. Mitochondria were solubilised by the additions of 0.1% Triton X-100 before assay of pyruvate carboxylase and phosphoenolpyruvate carboxykinase, and all activities were measured at 37°C. Blood glucose was measured in samples obtained at the time of sacrifice by the spectrophotometric method of Slien [11].

3. Results

3.1. *Enzyme activity following birth*

Measurement of enzyme activities at time of birth in guinea-pig kidney cortex shows a number of important facts (table 1). First of all, there is an extremely low activity of the two key mitochondrial enzymes in the gluconeogenic pathway. Pyruvate carboxylase and phosphoenolpyruvate carboxykinase are both present at below ten percent of the activity found in the adult animal. The activities of both of these enzymes rise slowly during the first three days of life, so that by two weeks the phosphoenolpyruvate carboxykinase has risen to 32% of the adult value and the pyruvate carboxylase to 63% of the adult value. On the other hand, the cytosolic phosphoenolpyruvate carboxykinase, though very low in activity at birth, undergoes a dramatic increase in the first 12 h of life and then suffers a slight decline only to achieve the adult level again at 2 weeks of life. Glucose 6-phosphatase activity is at 19% of the adult activity at birth and shows a similar slow rise to that seen with pyruvate carboxylase to reach 61% of adult activity by two weeks of life. Fructose 1,6-diphosphatase is the enzyme to show least change in the neonatal period, there being no significant difference between the newborn and the adult levels. Determination of blood glucose in samples taken at the time of sacrifice showed that these animals at no time become hypoglycaemic in the neonatal period.

4. Discussion

The gluconeogenic pathway is present both in liver and kidney cortex, but evidence from studies in the rat indicate that there is a temporal difference in the development of gluconeogenic capacity in the two tissues [5,12]. Glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphoenolpyruvate carboxykinase (mitochondrial) and pyruvate carboxylase are all present in the liver at birth in the guinea-pig [6], and the ability to carry out gluconeogenesis from lactate and alanine has recently been demonstrated in the perfused livers of prematurely delivered guinea-pigs [13]. Newborn rats, on the other hand, are able to carry out gluconeogenesis only at very slow rates, and this is said to result from the virtual absence of phosphoenolpyruvate carboxykinase in the livers of these animals [14]. The capacity for renal gluconeogenesis in the newborn rat, on the other hand, is acquired at birth and is demonstrable in tissue taken from the pre-term foetus [5].

The results presented here indicate that the neonatal guinea-pig kidney is poorly equipped to carry out gluconeogenesis at an appreciable rate. Pyruvate

Table 1
Activity of gluconeogenic enzymes in guinea-pig kidney cortex following birth

	Glucose 6-Phosphatase	Fructose 1,6-diphosphatase	Phosphoenolpyruvate carboxykinase		Pyruvate Carboxylase
			Mitochondrial	Cytosolic	
	(μmoles/g tissue/min)				
0 h	0.74 ± 0.07	0.96 ± 0.15	0.07 ± 0.01	0.03 ± 0.01	0.055 ± 0.008
2 h	0.69 ± 0.04	1.19 ± 0.16	0.13 ± 0.05	0.13 ± 0.02	0.080 ± 0.020
4 h	0.71 ± 0.08	1.30 ± 0.11	0.20 ± 0.04	0.27 ± 0.04	0.125 ± 0.032
6 h	0.74 ± 0.14	1.20 ± 0.20	0.24 ± 0.06	0.26 ± 0.06	0.163 ± 0.050
12 h	0.67 ± 0.09	1.19 ± 0.11	0.24 ± 0.05	0.44 ± 0.05	0.149 ± 0.047
24 h	0.99 ± 0.17	1.11 ± 0.13	0.25 ± 0.08	0.31 ± 0.05	0.187 ± 0.042
48 h	0.105 ± 0.14	1.63 ± 0.22	0.21 ± 0.06	0.23 ± 0.07	0.299 ± 0.059
72 h	1.30 ± 0.06	1.31 ± 0.10	0.24 ± 0.04	0.22 ± 0.01	0.322 ± 0.047
1 week	2.25 ± 0.2	1.30 ± 0.41	0.26 ± 0.05	0.31 ± 0.05	0.570 ± 0.071
2 weeks	2.39 ± 0.16	1.32 ± 0.10	0.35 ± 0.04	0.39 ± 0.07	0.611 ± 0.093
Adult	3.92 ± 0.31	1.10 ± 0.11	1.08 ± 0.09	0.46 ± 0.03	0.971 ± 0.034

Estimations were carried out as described in the methods section using six animals for each time point. Values are means + S.E.M.

carboxylase and both mitochondrial and cytosolic components of phosphoenolpyruvate carboxykinase have low activity at birth. Furthermore, the activities of pyruvate carboxylase and mitochondrial phosphoenolpyruvate carboxykinase are elevated to adult levels only over a relatively long period, while cytosolic phosphoenolpyruvate carboxykinase rises to adult levels within one day post-partum. The actual adult levels of these in enzymes in guinea-pig kidney cortex are appreciable and compare well with the levels found in adult guinea-pig liver on an activity/weight basis.

With the knowledge that gluconeogenic enzymes in newborn guinea-pig liver achieve adult levels within three days following birth [6], we conclude that the mechanisms governing the postnatal induction of these enzymes in liver and kidney are substantially different.

Acknowledgements

This research was supported by Medical Research Council of Canada and the Weston Foundation.

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