STIMULATION OF RAT KIDNEY PHOSPHOENOLPYRUVATE CARBOXYKINASE ACTIVITY IN EXPERIMENTAL LIVER DISEASE INDUCED BY GALACTOSAMINE

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1. Introduction

The administration of D-galactosamine hydrochloride to rats induces histological modifications which are closely related to acute viral hepatitis [1, 2]. Depletion of liver glycogen [3] and inhibition of gluconeogenesis [4, 5] are two important and related features among the biochemical effects involved in this kind of experimental liver damage. All of the so-called "key-gluconeogenic enzymes" show a significant decrease in their activity in these conditions [4], being specially striking the decrease in phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (PEPCK) [5].

Niederland et al. showed that kidneys of partially hepatectomized rats synthetize more glucose both *in vivo* and *in vitro* than the sham-operated animals, and that the activity of glucose-6-phosphatase and of fructose-1,6-diphosphatase increase during the enhanced renal gluconeogenesis [6]. Therefore it seemed interesting to investigate the metabolic response of kidney cortex from rats in which experimental liver damage was induced by administration of galactosamine.

In this paper we report the effect of galactosamine treatment on the gluconeogenic ability and PEPCK activity in rat liver and kidney. In order to elucidate the mechanism of the renal response in experimental liver damage, renal PEPCK activity has been also measured in animals administered with sodium bicarbonate or glucose.

2. Experimental

Female rats of the Wistar strain weighing 150–200 g were used. D(+)-Galactosamine hydrochloride (0.5–0.75 g/kg body weight) was administered as a neutral solution by a single intraperitoneal injection. The rats were sacrificed by cervical dislocation 24 hr after galactosamine administration. The animals exhibiting hepatitis (more than 90%) were used. Sodium bicarbonate was intragastrically administrated to the rats three times in 24 hr (10 ml of a 200 mM solution each time). Glucose was administered in the same way, the solution being 5.5 mM. Cycloheximide (1 mg per rat) was intraperitoneally injected 5 hr and 2.5 hr before killing. All the treatments were substituted by saline solution in the controls.

Liver gluconeogenic capacity was measured by perfusion with 10 mM L-lactate. The perfusion method has been described by Hems et al. [7]. The perfusate consisted of Krebs–Henseleit physiological saline [8], bovine serum albumin powder fraction V and washed human red cells stored 30 days at 4°C in citrate–dextrose anticoagulant solution. Glucose production by renal cortical slices was estimated according to Kreös et al. [9] by incubating the washed cells in saline medium to which L-lactate has been added in a 10 mM concentration, at 40°C for 1 hr, with $O_2 + CO_2$ (95:5) as the gas phase. Glucose was determined by the glucose oxidase method [9, 10].

The PEPCK activity was assayed spectrophotometrically in the direction of oxalacetate synthesis as previously described [11].

3. Results and discussion

Confirming previous works [4, 5], liver PEPCK activity in animals with liver damage induced by galactosamine was smaller than that of the nontreated animals (table 1). The drop was more pronounced in starved than in well fed rats (29 and 62 % of the control values, respectively). The results obtained in starved rats are in good agreement with those obtained by Record et al. [5], although our values are somewhat higher, probably because the starvation was more severe in our experiments (48 hr). Also the decreases in glucose-6-phosphatase and fructose-1,6-diphosphatase activities reported by Monier and Wagle in animals with experimental liver damage were more pronounced in starved animals [4].

Gluconeogenesis from L-lactate was lowered in the perfused liver of treated animals (table 1) in a similar extent as reported by Record et al. [5]. The decrease in the liver gluconeogenic ability was, however, considerably smaller than the drop in PEPCK activity which occured in starvation (66 and 29% of the normal values, respectively).

Whereas in starved animals the changes in renal PEPCK activity produced by galactosamine were not significant ($P < 0.1$), there was a 2-fold increase in the enzymatic activity in well-fed animals treated with galactosamine (table 1). The production of glucose by kidney cortex slices was also enhanced in well-fed animals ($P < 0.01$), although in a lesser extent (125% of control values) than PEPCK activity. According with the slight decrease observed in the enzymatic activity, renal gluconeogenesis was also poorly diminished ($P < 0.05$).

It is now well established that PEPCK activity and gluconeogenic capacity are enhanced in rat kidney in metabolic acidosis [12–17]. A similar response takes place in exercised animals likely because of the large quantities of lactate released by muscle in these conditions [11, 18]. Record et al. described that in experimental liver disease induced by galactosamine some increase in blood lactate occurs [5]. Hence, the possibility existed that the increase in renal PEPCK activity produced in the latter conditions could be related to the accumulation of lactate in plasma. As can be seen in table 2, the administration of sodium bicarbonate to the well-fed rats treated with galactosamine prevented only slightly the increase in renal PEPCK activity which occurs normally in these conditions. Moreover, it must be taken into account that sodium bicarbonate also depressed the values of the enzymatic activity in nontreated animals. Therefore, the increase of PEPCK activity in this kind of liver failure seems not to be influenced mainly by metabolic acidosis.

Another clear difference between the enhancement of kidney PEPCK activity in metabolic acidosis and in galactosamine induced hepatitis was evidenced by the effect of cycloheximide on the enzyme synthesis. As shown in table 3, the treatment with cycloheximide completely counteracted the enhancement of renal

Table 1

PEPCK activity and gluconeogenic ability of liver and kidney from rats treated with galactosamine.

Experimental conditions	PEPCK activity*		Gluconeogenic ability	
	Liver	Kidney	Liver**	Kidney***
Controls				
Well fed	1.51 ± 0.07 (6)	2.32 ± 0.10 (4)	–	107.5 ± 3.6 (4)
Starved (48 hr)	2.56 ± 0.11 (6)	6.51 ± 0.22 (5)	1.15 ± 0.06 (4)	224.0 ± 1.6 (4)
Galactosamine-treated				
Well fed	0.94 ± 006 (10)	4.53 ± 0.24 (9)	–	134.5 ± 2.1 (7)
Starved (48 hr)	0.76 ± 0.03 (8)	5.86 ± 0.38 (8)	0.77 ± 0.11 (4)	211.2 ± 3.9 (4)

* PEPCK activity is expressed in nmoles of oxalacetate formed at 30°C per min per g liver wet wt.
** Liver gluconeogenic ability is expressed in μmoles of glucose produced per min per g liver wet wt.
*** Kidney gluconeogenic ability is expressed in μmoles of glucose produced per hr per g kidney cortex dry wt.

The results are given as means ± S.E.M., with the number of observations in parentheses. Experimental details are given in the text.

Table 2

Effect of sodium bicarbonate and glucose administration on liver and kidney PEPCK activity from rats treated with galactosamine.

Tissue	Control	Control + $NaHCO_3$	Control + Glucose	Galactosamine-treated	Galactosamine-treated + $NaHCO_3$	Galactosamine-treated + Glucose
Liver	1.65 ± 0.09 (6)	1.56 ± 0.16 (7)	1.52 ± 0.16 (7)	0.86 ± 0.12 (7)	0.80 ± 0.07 (7)	0.85 ± 0.05 (9)
Kidney	2.26 ± 0.16 (6)	1.79 ± 0.11 (7)	2.72 ± 0.30 (7)	4.26 ± 0.28 (4)	3.80 ± 0.30 (7)	5.78 ± 0.22 (10)

PEPCK activity is expressed in nmoles of oxalacetate formed at 30°C per min per g liver wet wt. The results are given as means ± S.E.M. with the number of observations in parentheses.

PEPCK activity in galactosamine treated rats, indicating that this enhancement is related to de novo synthesis of the enzyme. However, the increase in renal PEPCK activity in response to metabolic acidosis is not due to a new synthesis of enzyme but to a change of the protein already present which alters its sensibility to the degradation [19–21].

Niederland et al. showed that the lack of glucose after partial hepatectomy is the direct stimulus which achieves the increase in kidney gluconeogenesis [6]. In order to evaluate if a similar lack of glucose is the primary stimulator of PEPCK activity in our experimental conditions, we also studied the effect of glucose administration to galactosamine-treated rats. The results given in table 2 show that the administration of glucose did not prevent the rise in kidney PEPCK activity brought about by galactosamine treatment and that even the values obtained were somewhat higher than those of the controls.

Whatever, the mechanism by which renal PEPCK activity and gluconeogenesis increase in experimental liver disease induced by galactosamine, these findings are of physiological interest because they point out that the kidney can play a key role in glucose homeostasis when the liver functional capacity diminishes. In addition to the usefulness of experimental liver disease induced by galactosamine as a model of viral hepatitis, this experimental condition seems to be also a valuable tool to study the relative quantitative role of liver and kidney in total gluconeogenesis.

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Table 3

Effect of cycloheximide on the stimulation of renal PEPCK activity in rat treated with galactosamine.

	Control	Cycloheximide	Galactosamine	Galactosamine + Cycloheximide
Renal PEPCK activity	2.15 ± 0.12 (4)	2.22 ± 0.14 (4)	3.72 ± 0.10 (4)	2.32 ± 0.13 (5)

PEPCK activity is expressed in nmoles of oxaloacetate formed at 30°C per min per g liver wet wt. The results are given as means ± S.E.M. with the number of observations in parentheses.

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