HETEROGENEOUS RECIPROCAL LOCALIZATION OF FRUCTOSE-1,6-BIS-PHOSPHATASE AND OF GLUCOKINASE IN MICRODISSECTED PERIPORTAL AND PERIVENOUS RAT LIVER TISSUE

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Received 9 September 1977
Revised version received 21 September 1977

1. Introduction

Liver and kidney catalyze glycolysis as well as gluconeogenesis [1–3]. In isolated liver cell suspensions the antagonistic processes were shown to occur simultaneously, the net process being dependent on substrate concentrations [4]. This mode of action of liver cells and the histochemically determined heterogeneous distribution of glucose-6-phosphatase (G6Pase) [5] and of glycogen metabolism [6,7] between periportal+ and perivenous+ zones of liver parenchyma led to the proposal of a metabolic zonation of the organ [4,5]. It was suggested that in the periportal zone glucose formation by gluconeogenesis and glycogenolysis might be the predominant process, while in the perivenous zone glycolysis linked to liponeogenesis should prevail. Such a zonation would be analogous to kidney cortex heterogeneity, gluconeogenesis being located in the proximal and glycolysis in the distal tubules [9–11]. The concept of a metabolic zonation of liver parenchyma was then further strengthened by demonstrating with the microdissection technique [12] that the glucogenic enzymes phosphoenolpyruvate carboxykinase [13] and glucose-6-phosphatase [14] were predominantly located in the periportal and the glycolytic pyruvate kinase [13] in the perivenous zone.

In the present investigation two other key enzymes of carbohydrate metabolism were quantitatively determined in periportal and perivenous liver tissue separated by microdissection. In support of the model the glucogenic fructose-1,6-bisphosphatase (FBPase) was found preferentially in the periportal and the glycolytic glucokinase (GK) in the perivenous zone.

2. Methods

Enzymes, coenzymes and substrates were from Boehringer GmbH, D-68 Mannheim; all other chemicals were reagent grade and obtained from Merck AG, D 61 Darmstadt. Female Wistar rats (150–180 g) were maintained on a 12 h day-night rhythm. Livers were removed after a short in situ perfusion with 0.9% NaCl solution during anesthesia with 60 mg sodium pentobarbital/kg body wt. For the histochemical reaction 10 μm sections and for the microdissection 20 μm sections were prepared as described [5]. Lyophilization, microdissection and determination of sample dry weight were carried out according to Lowry and Passonneau [12]. Parallel sections with histochemical demonstration of G6Pase [15] were used for identification of periportal and perivenous zones. Homogenates of fresh liver (1:500) or of lyophilyzed liver sections (1:2000) were prepared in a glass homogenizer with 100 mM potassium phosphate buffer pH 7.8 containing cysteine 5 mM, mercaptoethanol 1 mM and bovine serum albumin 1 mg/ml for FBPase assays and with 20 mM Tris–HCl buffer,

+Periportal, around the terminal portal vessels, i.e., mainly zone 1 of the liver acinus; perivenous (pericentral), around the central vein, i.e., mainly zone 3 of the liver acinus [8]

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pH 7.8, containing KCl 120 mM, $MgCl_2$ 4 mM, mercaptoethanol 10 mM and bovine serum albumin 1 mg/ml for GK measurements.

Fructose-1,6-bisphosphatase and glucokinase were measured by fluorimetric determination of NADPH production in incubations of 250–600 ng tissue dry weight or equivalent amounts of homogenate. Fructose-1,6-bisphosphatase was linked with glucose-phosphate isomerase (GPI) and glucose-6-phosphate dehydrogenase (G6PDH); glucokinase was combined with G6PDH. Fructose-1,6-bisphosphatase assay: fructose-1,6-bisphosphate 0.1 mM, $MgSO_4$ 5 mM, $NADP^+$ 0.2 mM, EDTA 0.1 mM, mercaptoethanol 15 mM, bovine serum albumin 0.5 mg/ml, GPI 4 mU, G6PDH 2 mU, 10 μl 20 mM Tris–HCl buffer pH 7.5, 30°C, 10 min. Glucokinase assay: glucose 50 mM, ATP 5 mM, $MgCl_2$ 5 mM, $NADP^+$ 0.1 mM, KCl 120 mM, mercaptoethanol 15 mM, G6PDH 1 mU, 10 μl 20 mM Tris–HCl buffer, pH 8.0, 30°C, 20 min. In both assays the reaction was stopped and $NADP^+$ was destroyed by adding 30 μl 75 mM NaOH and incubating at 56°C for 15 min. After cooling in an icebath 150 μl freshly prepared 8 N NaOH containing 3 mM H_2O_2 were added. The mixture was then incubated at 60°C for 15 min (FBPase) or 45 min (GK) in order to oxidize the NADPH formed to $NADP^+$ and to increase $NADP^+$ fluorescence; the longer incubation with the GK-assay was necessary, because high glucose concentrations retard fluorescence formation [12]. Readings of 150 μl aliquots diluted with 150 μl water were made with a Schoeffel fluorometer using 360 nm as excitation and 450 nm as emission wavelength. Each series of measurements contained NADPH standards between 20 pmol and 100 pmol.

3. Results

3.1. *The enzyme assays*

The fructose-1,6-bisphosphatase and the glucokinase reaction were linear with time up to 30 min and with protein in the range of 50–800 ng and 100–1000 ng liver dry wt, respectively. Within the same liver the enzyme activity in homogenates of fresh and of lyophilized tissue was in the same range.

The specificity of the fructose-1,6-bisphosphatase assay was demonstrated by its sensitivity to AMP. Inhibition of over 90% of the activity by AMP [16] indicated that unspecific acid or alkaline phosphatases were of minor importance only. The specificity of the glucokinase test was ensured by its substrate dependences. No attempt was made to differentiate between hexokinase (high affinity for glucose, $K_m \sim 10^{-4}$ M) and glucokinase proper (low affinity for glucose, $K_m \sim 10^{-2}$ M) [17]. With the high glucose concentration used in the assay total glucose phosphorylating activity was determined, in the normal fed rat glucokinase makes up 75–80% of the total activity [17–19]

3.2. *The distribution of fructose-1,6-bisphosphatase (FBPase) and of glucokinase (GK) in periportal and perivenous liver tissue*

Having established, that the enzyme assays were specific and sensitive enough for measurements in less than 100 ng liver dry wt, FBPase and GK were determined in 250–600 ng (90–200 hepatocytes) microdissected periportal and perivenous liver tissue. The chosen sample sizes almost completely represented the histochemically marked periportal and perivenous zones of the liver acinus.

The specific activities of both FBPase and GK in the two zones and the periportal to perivenous activity ratio varied from one liver to other. Since there was this remarkable variation within a rat population both as to absolute activities and as to the gradients over the parenchyma not only the average but also the individual values have been documented (table 1). For FBPase the average ratio was 1.91 : 1 in fed and 1.45 : 1 in fasted rats (the ratios would be 2.1 : 1 and 1.5 : 1, if the FBPase measurements were corrected for the unspecific phosphatase, which cannot be inhibited by AMP); inversely, for GK the average ratio amounted to 1:1.97 in fed and 1:1.66 in fasted animals (table 1). After 24 h starvation the FBPase activity rose slightly in the periportal and in the perivenous zone, while the GK activity remained essentially unaltered (table 1).

4. Discussion

In this communication it was shown with a microdissection technique and microfluorimetric enzyme assays of high sensitivity, that in fed rats the gluconeogenic FBPase was approx. two-fold more active

Table 1
Fructose-1,6-bisphosphatase and glucokinase activity in periportal and perivenous liver tissue

Liver		Enzyme activity (μmol/min/g dry wt)			
		Periportal	Perivenous	*P*	Periportal / Perivenous
1. Fructose-1,6-bisphosphatase					
Fed	8	24.4 ± 3.9 (5)	12.2 ± 6.3 (3)	< 0.01	2.00 : 1
	9	28.6 ± 4.5 (4)	14.8 ± 1.0 (4)	< 0.0005	1.93 : 1
	10	41.5 (1)	12.7 ± 0.9 (3)		3.27 : 1
	13	15.9 ± 2.2 (5)	8.1 ± 0.7 (5)	< 0.0005	1.96 : 1
	14	24.0 ± 3.0 (7)	14.7 ± 1.9 (6)	< 0.0005	1.63 : 1
	Σ	23.9 ± 6.6 (22)	12.5 ± 3.6 (21)	< 0.0005	1.91 : 1
Fasted (24 h)	15	25.8 ± 7.0 (8)	18.0 ± 4.2 (7)	< 0.0125	1.42 : 1
	16	27.1 ± 10.7 (4)	16.7 ± 3.6 (5)	< 0.05	1.62 : 1
	17	27.6 + 10.3 (8)	19.7 + 4.4 (10)	< 0.025	1.40 : 1
	Σ	26.8 ± 8.7 (20)	18.4 ± 4.1 (22)	< 0.0005	1.45 : 1
2. Glucokinase					
Fed	1	9.6 ± 2.4 (4)	16.8 ± 2.4 (4)	< 0.005	1 : 1.75
	2	5.3 ± 1.0 (3)	15.1 ± 2.6 (3)	< 0.0025	1 : 2.85
	3	8.6 ± 1.4 (4)	17.8 ± 0.7 (3)	< 0.0005	1 : 2.07
	4	10.6 ± 2.4 (5)	16.6 ± 1.9 (5)	< 0.0025	1 : 1.57
	5	7.0 ± 1.9 (3)	12.8 ± 2.6 (3)	< 0.025	1 : 1.83
	6	7.4 ± 2.4 (5)	9.1 ± 1.4 (4)	< 0.15	1 : 1.23
	7	5.0 ± 0.7 (5)	10.1 ± 0.7 (5)	< 0.0005	1 : 2.02
	9	5.0 ± 3.6 (4)	10.8 ± 5.3 (4)	< 0.1	1 : 2.16
	10	4.8 ± 2.4 (4)	12.7 ± 1.9 (4)	< 0.0025	1 : 2.65
	11	7.2 ± 0.7 (6)	16.8 ± 0.7 (6)	< 0.0005	1 : 2.33
	12	10.3 ± 0.2 (2)	17.0 ± 1.2 (4)	< 0.025	1 : 1.65
	Σ	7.2 ± 2.6 (45)	14.2 ± 3.6 (45)	< 0.0005	1 : 1.97
Fasted (24 h)	15	7.1 ± 2.1 (5)	15.0 ± 6.0 (3)	< 0.025	1 : 2.11
	16	7.7 ± 0.8 (5)	10.2 ± 1.8 (4)	< 0.005	1 : 1.32
	17	9.9 ± 0.8 (6)	16.1 ± 2.8 (5)	< 0.025	1 : 1.63
	Σ	8.3 ± 1.8 (16)	13.8 ± 4.2 (12)	< 0.0005	1 : 1.66

Mean value ± standard deviation

Number of determinations in parentheses

Statistics: Student's *t*-test

in periportal than in perivenous zones and that inversely the glycolytic GK was about two-fold more active in perivenous than in periportal areas. Other key enzymes of carbohydrate metabolism also exhibit similar periportal/perivenous activity ratios. The glucogenic enzymes phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and glutamate–pyruvate transaminase showed ratios of 2.9 : 1 [13], 2.3 : 1 [14] and 2.8 : 1 [20] or 5 : 1 [21], respectively, while the glycolytic pyruvate kinase had a ratio of 1 : 2.3 [13].

In 24 h fasted animals the glucogenic FBPase activity was only slightly increased and the glycolytic GK activity was unchanged. The FBPase increase is in line with two previous reports [19,22] but in contrast with one other [23]. In agreement with the present study an unaltered GK activity after 24 h starvation was reported by some investigators [19,24,25], while a decreased activity was found by other [17,18,26]. At present there is no obvious explanation for these discrepancies. The periportal/perivenous activity gradients were decreased in the fasted state both for FBPase and GK. This decrease might have been expected since it had already been found for glucose-6-phosphatase, phosphoenolpyruvate carboxykinase and pyruvate kinase [13,14]. The change of the periportal to perivenous activity ratios upon starvation, so far observed with all enzymes tested [13,14], indicates a dynamic, functional rather than a static, structural zonation [14]. The results of the present investigation are consistent with the proposal [4,5,27] that gluconeogenesis and glycolysis may be preferentially located in different zones of liver parenchyma similar to the spatial separation of the two processes in kidney [9–11].

The periportal to perivenous activity ratios in liver acini are only in the range of 2 : 1 – 5 : 1; the proximal to distal activity gradients in kidney tubules are much larger in the range of 7 : 1 – 30 : 1 [9–11]. It has been suggested [14] that these differences may reflect the different physiological functions of liver and kidney in carbohydrate metabolism.

It has also been pointed out previously [14,27] that the relatively low hepatic periportal to perivenous activity ratios should be functionally significant. Two arguments were advanced: First, if the ratio for a glucogenic enzyme were only 2 : 1 and for a glycolytic enzyme 1 : 2, the overall enzyme equipment of the two zones would be different by a factor of 4; differences of this order of magnitude should be metabolically meaningful. Second, if – as could be expected under some conditions – all three irreversible steps of gluconeogenesis and glycolysis would contribute to the rate limitation, this difference factor of the two zones should be further increased. A third argument may be added: It is well feasible that different metabolite and effector concentrations are being maintained in the two zones. This might intensify the proposed functional differences between the periportal and the perivenous areas. Therefore, the present results can be regarded as support for the hypothesis of a 'Metabolic Zonation' of liver parenchyma.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Dr K. Decker for valuable discussions and Mrs F.-Chr. Hebel and R. Heussner for their skillful assistance.

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