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Zonation of fatty acid metabolism in rat liver

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Fatty acid metabolism was studied in periportal and perivenous hepatocytes isolated by the method of Chen & Katz [Biochem. J. (1988) **255**, 99–104]. The rate of fatty acid synthesis and the activity of acetyl-CoA carboxylase were markedly enhanced in perivenous hepatocytes as compared with periportal cells. However, the response of these two parameters to short-term modulation by cellular effectors such as the hormones insulin and glucagon, the phorbol ester 4β-phorbol 12β-myristate 13α-acetate and the xenobiotics ethanol and acetaldehyde was similar in the two zones of the liver. In addition, perivenous hepatocytes showed a higher capacity of esterification of exogenous fatty acids into both cellular and very-low-density-lipoprotein lipids. Nevertheless, no difference between the two cell sub-populations seemed to exist in relation to the secretion of very-low-density lipoproteins. On the other hand, the rate of fatty acid oxidation was increased in periportal cells. This could be accounted for by a higher activity of carnitine palmitoyltransferase I and a lower sensitivity of this enzyme to inhibition by malonyl-CoA in the periportal zone. No differences were observed between periportal and perivenous hepatocytes in relation to the short-term response of fatty acid oxidation and carnitine palmitoyltransferase I activity to the cellular modulators mentioned above. In conclusion, our results show that: (i) lipogenesis is achieved at higher rates in the perivenous zone of the liver, whereas the fatty-acid-oxidative process occurs with a certain preference in the periportal area of this organ; (ii) the short-term response of the different fatty-acid-metabolizing pathways to cellular effectors is quantitatively similar in the two zones of the liver.

INTRODUCTION

Evidence has accumulated during the last decade supporting the hypothesis of liver zonation. Contrasting with a virtually uniform morphology, parenchymal liver cells have been shown to be rather heterogeneous with respect to ultrastructure, metabolic features and enzymic equipment (Jungermann, 1986; Gumucio, 1989). Thus two different zones may be distinguished within the liver acinus, namely an afferent periportal area and an efferent perivenous region (Jungermann, 1986; Gumucio, 1989).

A number of investigations have focused on the study of carbohydrate metabolism in these two hepatic zones. Based on microdissection techniques, Jungerman and co-workers proposed a few years ago that periportal hepatocytes are predominantly gluconeogenic, whereas perivenous cells are mostly glycolytic (Jungermann & Katz, 1982; Jungermann, 1986). More recently, methods for specific isolation of hepatocytes from the two acinar zones have been reported (Lindros & Penttilä, 1985; Quistorff, 1985; Chen & Katz, 1988). The use of this technique reveals that both gluconeogenesis and glycogen synthesis occur preferentially in periportal cells, whereas no zonation of glycolysis seems to exist (Chen & Katz, 1988, and references cited therein). Another recent report on the distribution of fructose 2,6-bisphosphate is also in agreement with a lack of zonation of glycolysis in rat liver (Wals *et al.*, 1988).

In contrast with this knowledge on the zonation of carbohydrate metabolism, the distribution of fatty acid metabolism within the liver acinus has not been completely determined (see Tosh *et al.*, 1988). Thus, in the present study we isolated periportal and perivenous hepatocytes by the digitonin/collagenase-perfusion method described by Chen & Katz (1988) and investigated whether fatty acid metabolism is zonated in rat liver. Our results show that lipogenesis takes place predominantly in the perivenous zone, whereas fatty acid oxidation preferentially occurs in the periportal area. Nevertheless, this heterogenous distribution of the different fatty-acid-metabolizing pathways is not accompanied by variations in the responses of these processes to short-term modulation by cellular effectors.

MATERIALS AND METHODS

Materials

^3H_2O (5 Ci/ml), [1-^{14}C]palmitic acid (58 Ci/mol), [U-^{14}C]palmitic acid (403 Ci/mol), L-[*Me*-^{3}H]carnitine (71 Ci/mmol) and [1-^{14}C]acetyl-CoA (54.3 Ci/mol) were supplied by Amersham International (Amersham, Bucks., U.K.). Acetyl-CoA, malonyl-CoA, butyryl-CoA, palmitoyl-CoA, L-carnitine, bovine serum albumin (fraction V; essentially fatty acid-free), L-carnitine, digitonin, PMA, pig insulin and glucagon, collagenase (type I), glycerol kinase, glycerol-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase (type XXIV) and lactate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents and solvents were analytical-reagent grade from Sigma, Merck (Darmstadt, Germany), Boehringer (Mannheim, Germany) and Aldrich (Steinheim, Germany).

Abbreviations used: VLDL, very-low-density lipoproteins; PMA, 4β-phorbol 12β-myristate 13α-acetate; CPT, carnitine palmitoyltransferase (EC 2.3.1.21); CPT-I and CPT-II, overt and latent form of CPT activity respectively.

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Hepatocyte isolation

Periportal and perivenous hepatocytes were isolated from male Wistar rats (250–300 g) by the procedure of Chen & Katz (1988). This method was chosen because it seems to allow preparations of periportal and perivenous hepatocytes quite capable of reproducible rates of glycogen synthesis and glycolysis. Since lipogenesis is markedly depressed just after the isolation procedure, hepatocytes were incubated for 15 min at 37 °C in a metabolic gyratory shaker and then filtered through nylon mesh, as recommended by Holland *et al.* (1984). Isolated cells were finally suspended in Krebs–Henseleit bicarbonate buffer supplemented with 10 mM-glucose. Cell viability, as determined by Trypan Blue exclusion, always exceeded 85% in the final hepatocyte suspension.

Hepatocyte incubations and fatty acid metabolism

Hepatocytes were incubated in Krebs–Henseleit bicarbonate buffer supplemented with 10 mM-glucose and 2% (w/v) defatted and dialysed bovine serum albumin. Incubations were carried out in a total volume of 2 ml in a gyratory metabolic shaker (85 rev./min) at 37 °C in 25 ml Erlenmeyer flasks which contained 4–6 mg of cellular protein/ml (Jurin & McCune, 1985). During incubation, the flasks were continuously gassed with O_2/CO_2 (19:1). Hepatocyte incubations were performed to monitor the rates of fatty acid synthesis, esterification and oxidation. In addition, part of the cells were incubated in radioisotope-free flasks and used for the determination of enzyme activities (see below).

(i) The rates of fatty acid synthesis *de novo* were determined by the addition of ^3H_2O (0.4 mCi/ml) to the cell incubations. After 60 min, reactions were stopped and total fatty acids were extracted as described by Tijburg *et al.* (1988).

(ii) The rates of fatty acid esterification were monitored by incubating hepatocytes with 0.4 mM-[U-^{14}C]palmitate (0.05 Ci/mol) bound to albumin. After 60 min, cells were transferred to centrifuge tubes and separated from the incubation medium (Janski & Cornell, 1980), from which very-low-density lipoproteins (VLDL) were isolated by gradient ultracentrifugation (Terpstra, 1985). The appearance of VLDL in the incubation medium had a lag phase of approx. 20 min, and so rates of VLDL secretion were calculated between 30 and 60 min of cell incubation. Both cellular and VLDL lipids were extracted by the method of Bligh & Dyer (1959). Lipids were separated by t.l.c. on silica-gel G plates with hexane/diethyl ether/formic acid (40:20:1, by vol.) as developing system. Individual bands were scraped off for radioactivity counting.

(iii) The rates of fatty acid oxidation were measured in cell incubations containing 0.4 mM-[1-^{14}C]palmitate (0.05 Ci/mol) bound to albumin and carried out for 30 min. Radiolabelled CO_2 and ketone bodies were quantified as described by Guzmán & Geelen (1988*a*). Total oxidation products were calculated as the sum of CO_2 plus total acid-soluble products. The latter routinely accounted for more than 85% of the total oxidation products (results not shown).

Acetyl-CoA carboxylase assay

The activity of acetyl-CoA carboxylase was measured in digitonin-permeabilized hepatocytes by determining the incorporation of radiolabelled acetyl-CoA into fatty acids in a reaction coupled to the fatty acid synthase reaction as described by Bijleveld & Geelen (1987). Assays were started by the addition of 100 μl of hepatocyte suspension to 100 ml of pre-heated (37 °C) iso-osmotic assay medium supplemented with digitonin. The final assay mixture contained 63 mM-Hepes (pH 7.5), 5 mM-glucose, 1.5 mM-$MgCl_2$, 0.5 mM-$MgSO_4$, 0.5 mM-citrate, 2.5 mM-EGTA, 1.25 mM-$CaCl_2$, 22.5 mM-$NaHCO_3$, 70.5 mM-NaCl, 2.0 mM-ATP, 0.5 mM-NADPH, 0.44 mM-dithioerythritol, 0.925% bovine serum albumin (defatted and dialysed), 0.062 mM-[1-^{14}C]acetyl-CoA (4 Ci/mol), 0.062 mM-butyryl-CoA, 64 μg of digitonin/mg of cellular protein and 3.2 munits of fatty acid synthase (1 munit = 1 μmol/min). Reactions were stopped with 0.1 ml of 10 M-NaOH, and samples were saponified by boiling for 1 h in the presence of 5 ml of 0.3 M-NaOH in 90% (v/v) methanol. After cooling and acidification with 0.2 ml of 12 M-HCl, fatty acids were extracted with light petroleum (b.p. 40–60 °C) (Bijleveld & Geelen, 1987).

Carnitine palmitoyltransferase I (CPT-I) assay

The activity of CPT-I was determined as the malonyl-CoA-sensitive incorporation of radiolabelled L-carnitine into palmitoylcarnitine, both in mitochondria isolated from intact hepatocytes and in cells permeabilized with digitonin. In the first case, mitochondria were isolated from hepatocyte incubations and enzyme activity was measured as described by Guzmán *et al.* (1987). In the second case, 100 μl of hepatocyte suspension was added to 100 μl of pre-heated (37 °C) iso-osmotic assay medium supplemented with digitonin. Further details of this enzyme assay are described elsewhere (Guzmán & Geelen, 1988*b*). The final assay mixture contained 12.5 mM-Tris/HCl (pH 7.4), 70 mM-sucrose, 5 mM-glucose, 32.5 mM-KCl, 12.5 mM-$NaHCO_3$, 60 mM-NaCl, 0.6 mM-KH_2PO_4, 0.6 mM-$MgSO_4$, 1.25 mM-$CaCl_2$, 1 mM-EDTA, 1 mM-dithioerythritol, 50 μM-palmitoyl-CoA, 0.5 mM-L-[*Me*-^{3}H]carnitine (3 Ci/mol), 0.5% bovine serum albumin (defatted and dialysed) and 40 μg of digitonin/mg of cellular protein. Reactions were stopped by the addition of either 1.0 ml (isolated mitochondria) or 0.3 ml (permeabilized cells) of 1 M-HCl, and [^{3}H]palmitoylcarnitine was extracted with butan-1-ol (Saggerson & Carpenter, 1986). In both assays, CPT-I activity always accounted for more than 90% of the total CPT activity experimentally determined (results not shown). Malonyl-CoA-insensitive CPT activity, representing both the latent form of CPT activity (CPT-II) as well as peroxisomal carnitine octanoyltransferase activity, was always subtracted from total CPT activity.

Other analytical methods

For the assay of marker enzyme activities, freshly isolated cells (5–10 mg of protein) were frozen in 2 ml of a medium containing 150 mM-KCl, 50 mM-Hepes (pH 7.5) and 5 mM-dithioerythritol. Thawed samples were homogenized, centrifuged at 12500 *g* for 15 min, and then supernatants were collected for enzyme assay (Chen & Katz, 1988). Marker enzyme activities were determined in parallel in biopsies of whole liver. Activities of alanine aminotransferase, lactate dehydrogenase and glutamate dehydrogenase were determined by standard spectrophotometric methods (Bergmeyer, 1983), and that of glucokinase was measured as described by Chen & Katz (1988). The activity of low-K_m hexokinase was deter-

mined exactly as for glucokinase, except that glucose concentration was 0.5 mM.

Cellular lipids were extracted and separated as described above. Triacylglycerols were measured as free glycerol with glycerol kinase and glycerol-3-phosphate dehydrogenase (Bergmeyer, 1983). Phospholipids were quantified by phosphorus analysis (Rouser *et al.*, 1966). Cholesterol esters were determined by the cholesterol esterase/cholesterol oxidase/peroxidase method, supplied in kit form by Boehringer.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Statistical analysis

Results shown represent the means ± S.D. of the numbers of animals of each group indicated in every case. Cell incubations and/or enzyme assays were always carried out in triplicate. Statistical analysis was performed by the unpaired Student *t* test.

RESULTS AND DISCUSSION

Characterization of hepatocytes

Periportal and perivenous hepatocytes were obtained by the technique of short-term digitonin perfusion, as described by Chen & Katz (1988), and further characterized according to the distribution pattern of several marker enzymes. As can be inferred from Table 1, the activities of alanine aminotransferase and lactate dehydrogenase were higher in the periportal zone, whereas glucokinase and glutamate dehydrogenase were more active in the perivenous region of the liver acinus. These results are in agreement with previous data from different laboratories (Lindros & Penttilä, 1985; Quistorff, 1985; Chen & Katz, 1988), indicating a selective enrichment in periportal and perivenous hepatocytes in the respective cell preparations. In addition, the low-K_m hexokinase activity in all the samples was insignificant (results not shown). This observation indicates the absence of sinusoidal cells from the cell preparations obtained after the isolation procedure.

Fatty acid synthesis

The rates of fatty acid synthesis *de novo* were studied in periportal and perivenous hepatocytes incubated with ^3H_2O. Fatty acid synthesis was markedly enhanced in perivenous hepatocytes as compared with periportal cells (Table 2). This is at odds with previous results obtained by Quistorff *et al.* (1986), who did not observe any difference between the two cell sub-populations. This discrepancy could be due to differences in the cell isolation procedure, as well as to the fact that those authors used hepatocytes from fasted rats, which display an extremely low lipogenic capacity (Geelen *et al.*, 1980).

Acetyl-CoA carboxylase is considered to be the rate-limiting step of fatty acid synthesis *de novo* in liver and extrahepatic tissues (Geelen *et al.*, 1980; Wakil *et al.*, 1983). Therefore we determined acetyl-CoA carboxylase activity in the two cell sub-populations. Acetyl-CoA carboxylase activity was measured in a permeabilized-cell system with radiolabelled acetyl-CoA as substrate in an assay coupled to the fatty acid synthase reaction (Bijleveld & Geelen, 1987). The use of this procedure avoids the accumulation of malonyl-CoA in the incubation medium, which inhibits acetyl-CoA carboxylase activity, and obviates any interference with mitochondrial enzymes (Bijleveld & Geelen, 1987, and references cited therein). In accordance with micro-dissection studies performed by Katz *et al.* (1983*a*,*b*, 1989), showing increased activities of acetyl-CoA carboxylase, ATP citrate lyase and fatty acid synthase in the perivenous zone of the liver (for fatty acid synthase, after distinct induction procedures), acetyl-CoA carboxylase activity was observed to be enhanced in perivenous hepatocytes as compared with periportal cells (Table 2). Moreover, the magnitude of this increase was similar to that of the fatty-acid-synthesizing process, supporting the general view that acetyl-CoA carboxylase plays a major regulatory role in fatty acid synthesis *de novo*. Hence perivenous hepatocytes seem to be better equipped to perform the fatty-acid-synthesizing process, possibly in relation to their lower capacity to carry out gluconeogenesis (Quistorff, 1985; Chen & Katz, 1988). Nevertheless, it has recently been reported that both the mass and the specific activity of acetyl-CoA carboxylase, ATP citrate lyase and fatty acid synthase are considerably higher in the periportal than in the perivenous zone of the liver, as determined by the dual-digitonin perfusion method (Evans *et al.*, 1989). The reasons for the different distribution patterns obtained by the use of these three distinct experimental approaches (i.e. micro-dissection and digitonin/collagenase perfusion on the one hand, and dual-digitonin perfusion on the other hand) are not understood.

In addition to the absolute values of both the rate of fatty acid synthesis and the activity of acetyl-CoA

Table 1. Activities of marker enzymes in periportal and perivenous hepatocytes

Enzyme activities were determined both in hepatocytes isolated from the periportal (PP) or the perivenous (PV) zone of the liver and in liver biopsies as described in the Materials and methods section. Enzyme activities are expressed as nmol/min per mg of cellular protein. The cell/liver ratio denotes the cellular enzyme activity relative to that determined in a sample from the corresponding liver. [a]Significantly different ($P < 0.01$) from the cell/liver ratio in the periportal zone.

	PP ($n = 8$)		PV ($n = 8$)		
Enzyme	Enzyme activity	Cell/liver ratio	Enzyme activity	Cell/liver ratio	PP/PV
Alanine aminotransferase	112.5 ± 14.8	1.47 ± 0.15	69.4 ± 8.7	0.84 ± 0.10[a]	1.75
Lactate dehydrogenase	1507.5 ± 166.3	1.32 ± 0.08	1128.6 ± 57.9	0.93 ± 0.07[a]	1.42
Glucokinase	18.1 ± 1.9	1.53 ± 0.12	29.2 ± 4.1	2.39 ± 0.26[a]	0.64
Glutamate dehydrogenase	880.7 ± 73.0	1.05 ± 0.12	1191.2 ± 87.6	1.53 ± 0.17[a]	0.69

Table 2. Activity of acetyl-CoA carboxylase and rates of fatty acid synthesis in periportal and perivenous hepatocytes

Hepatocytes were isolated from the periportal (PP) or the perivenous (PV) zone of the liver and preincubated in the presence of the additions indicated. After 30 min, part of the cells were used for measurement of acetyl-CoA carboxylase activity. The rest of the cells were used for determination of the rates of fatty acid synthesis. For incubations with no additions, values of acetyl-CoA carboxylase activity are expressed as nmol of acetyl-CoA fixed/min per mg of cellular protein, whereas those of the rates of fatty acid synthesis are expressed as nmol of acetyl units/h per mg of cellular protein. The effects on these two parameters induced by incubation with the different cellular effectors are expressed as percentages of incubations with no additions. [a]Significantly different ($P < 0.01$) from periportal hepatocytes.

Additions	Activity of acetyl-CoA carboxylase			Rate of fatty acid synthesis		
	PP ($n = 8$)	PV ($n = 8$)	PP/PV	PP ($n = 9$)	PV ($n = 9$)	PP/PV
None	0.49±0.07	0.73±0.09[a]	0.67	55.9±7.1	89.5±10.2[a]	0.63
Change caused by:						
Insulin (85 nM)	128±10	131±11	0.98	134±7	138±12	0.97
Glucagon (10 nM)	69±7	63±13	1.10	58±12	65±6	0.89
PMA (1 μM)	131±9	138±4	0.95	132±8	141±15	0.94
Ethanol (20 mM)	152±23	168±17	0.90	152±10	177±18	0.86
Acetaldehyde (5 mM)	150±11	143±20	1.05	161±21	154±17	1.05

Table 3. Rates of fatty acid esterification and VLDL secretion in periportal and perivenous hepatocytes

Hepatocytes were isolated from the periportal (PP) or the perivenous (PV) zone of the liver and incubated in the presence of albumin-bound 0.4 mM-[U-^{14}C]palmitate. After 1 h, cells were separated from the incubation medium, and radiolabelled triacylglycerols, phospholipids and cholesterol esters were determined both in the cellular and in the VLDL fraction. Results are expressed as nmol of palmitate into lipid/h per mg of cellular protein. Significantly different from periportal hepatocytes: [a]$P < 0.05$; [b]$P < 0.01$.

Fraction	Lipid	Fatty acid into lipid		
		PP ($n = 8$)	PV ($n = 8$)	PP/PV
Cell	Triacylglycerols	6.44±0.75	10.16±1.83[b]	0.63
	Phospholipids	5.52±0.36	7.26±0.93[a]	0.76
	Cholesterol esters	0.29±0.05	0.41±0.05[a]	0.71
VLDL	Triacylglycerols	1.35±0.28	2.03±0.37[b]	0.67
	Phospholipids	0.38±0.04	0.49±0.04[a]	0.78
	Cholesterol esters	0.10±0.01	0.13±0.01[a]	0.77

carboxylase, the short-term modulation of these two parameters by the polypeptide hormones insulin and glucagon, the phorbol ester PMA and the xenobiotics ethanol and acetaldehyde was studied in periportal and perivenous hepatocytes. For this purpose, acetyl-CoA carboxylase activity was determined in the permeabilized-cell system, since the alterations induced by cellular effectors on the enzyme may be lost (at least in part) during cell fractionation (Bijleveld & Geelen, 1987; Tijburg *et al.*, 1988). As can be inferred from Table 2, insulin, PMA, ethanol and acetaldehyde increased the rate of fatty acid synthesis *de novo* and the activity of acetyl-CoA carboxylase in parallel, whereas glucagon had the opposite effect (Bijleveld & Geelen, 1987; Tijburg *et al.*, 1988). The effect of acetaldehyde on acetyl-CoA carboxylase is new, and may be related to that exerted on the enzyme by ethanol (Tijburg *et al.*, 1988). Nevertheless, all these effects were not significantly different between periportal and perivenous hepatocytes, in line with the observation by Tosh *et al.* (1988) that the short-term modulation by glucagon of gluconeogenesis and ketogenesis is similar in both cell sub-populations.

Fatty acid esterification and lipid secretion

As shown in Table 3, the incorporation of radiolabelled palmitate into cellular triacylglycerols, phospholipids and cholesterol esters was higher in perivenous than in periportal hepatocytes. In addition, the zonation of triacylglycerol synthesis seems to be more pronounced than that of phospholipid synthesis, supporting the notion that the latter is a more constitutive pathway, owing to the essential role of phospholipids as biomembrane constituents (cf. Tijburg *et al.*, 1988). Thus two major anabolic pathways occur, with a certain separation within the liver acinus: lipogenesis takes place preferentially in the perivenous zone (Tables 2 and 3), whereas glycogen synthesis is mainly performed by the periportal area (Chen & Katz, 1988).

Isolated hepatocytes constitute a suitable model for the study of hepatic VLDL secretion. These cells secrete VLDL at rates comparable with those taking place *in vivo*, this process retaining the sensitivity to both short- and long-term regulation (see, e.g., Beynen *et al.*, 1981; Gibbons & Pullinger, 1987). Hence we monitored the

rates of [^{14}C]palmitate incorporation not only into cellular lipids, but also into extracellular VLDL. The higher lipogenic capacity of perivenous hepatocytes was also reflected in the amount of lipids secreted by this cell subpopulation into the incubation medium as VLDL, i.e. VLDL lipids were synthesized at a higher rate in perivenous than in periportal hepatocytes (Table 3). Two observations suggest that this zonation of VLDL synthesis is simply due to a 'push' effect produced by the lipid (substrate) burden in the perivenous zone of the liver, and not to an enhanced capacity of perivenous hepatocytes to carry out the secretory mechanism (i.e. assembly plus exocytosis). (i) For the three lipid classes examined, the ratio VLDL lipid/cellular lipid was not significantly different between perivenous and periportal hepatocytes (see Table 3); (ii) in a series of experiments, hepatocyte lipids were prelabelled by incubating the cells in the presence of [^{14}C]palmitate. Cells were subsequently washed and further incubated in a radioisotope-free medium. However, no differences were observed between periportal and perivenous hepatocytes in the rate of appearance of labelled triacylglycerols, phospholipids and cholesterol esters in the incubation medium in relation to the label incorporated into those cellular lipids (results not shown). Therefore, perivenous cells incorporate more fatty acids into cellular lipids. This lipid load is secreted into the extracellular medium as VLDL, so that lipids do not accumulate in the perivenous area of the liver, as indicated by the lipid content of the two cell sub-populations (Table 4).

Table 4. Lipid content of periportal and perivenous hepatocytes

Cellular levels of triacylglycerols, phospholipids and cholesterol esters were determined in hepatocytes isolated from the periportal (PP) or the perivenous (PV) zone of the liver. Results are expressed as nmol of lipid/mg of cellular protein.

Lipid	PP ($n = 8$)	PV ($n = 10$)	PP/PV
Triacylglycerols	77.8±6.1	83.6±5.0	0.93
Phospholipids	188.0±7.5	199.2±11.3	0.94
Cholesterol esters	5.4±0.4	5.8±0.3	0.93

Fatty acid oxidation

The rates of fatty acid oxidation in periportal and perivenous hepatocytes are shown in Table 5. Periportal hepatocytes were able to carry out the fatty-acid-oxidative process at a slightly higher rate than perivenous cells. This was reflected both in the acid-soluble and in the CO_2 components of total oxidation product. Thus the rates of acid-soluble product formation were 32.37±3.26 and 24.08±3.13 nmol of palmitate into product/h per mg of cellular protein in periportal and perivenous hepatocytes respectively, whereas those of CO_2 production were 5.85±0.79 and 4.58±0.37 nmol of palmitate into product/h per mg of cellular protein (in both cases, $P < 0.05$ by the paired t test). The same trend was previously observed by Tosh *et al.* (1988) in relation to ketogenesis, although the values they obtained were not significantly different between periportal and perivenous hepatocytes incubated in the absence of glucagon. Similarly, microdissection studies performed by Katz *et al.* (1983*a*) showed that the activity of 3-hydroxyacyl-CoA dehydrogenase, one of the enzymes of fatty acid β-oxidation, was slightly higher in the periportal than in the perivenous zone of the liver. Nevertheless, it is curious that periportal cells exhibit a higher rate of ketogenesis but a decreased activity of 3-hydroxybutyrate dehydrogenase (Katz *et al.*, 1983*a*) and a lower 3-hydroxybutyrate/acetoacetate production ratio (Tosh *et al.*, 1988).

CPT-I is considered to play a regulatory role in the transfer of long-chain fatty acids into the mitochondrial matrix, in both liver (McGarry & Foster, 1980) and extrahepatic tissues (Saggerson, 1986). Both short-term (Guzmán & Geelen, 1988*b*) and long-term (Zammit, 1986) regulation of CPT-I activity has been shown to occur in rat liver. Moreover, CPT-I seems to catalyse the rate-limiting step of the fatty acid oxidative process (Stephens & Harris, 1987, and references cited therein), although additional control points might exist in the regulation of this metabolic pathway (Zammit, 1984). Hence, we determined the activity of this enzyme in

Table 5. Activity of CPT-I and rates of fatty acid oxidation in periportal and perivenous hepatocytes

Hepatocytes were isolated from the periportal (PP) or the perivenous (PV) zone of the liver and preincubated in the presence of the additions indicated. After 30 min, part of the cells were used for measurement of CPT-I activity. The rest of the cells were used for determination of the rates of fatty acid oxidation. In the case of incubations with no additions, values of CPT-I activity are expressed as nmol of palmitoylcarnitine/min per mg of cellular protein, whereas those of the rates of fatty acid oxidation are expressed as nmol of palmitate into total oxidation product/h per mg of cellular protein. The effects on these two parameters induced by incubation with the different cellular effectors are expressed as percentages of incubations with no additions. [a]Significantly different ($P < 0.05$) from periportal hepatocytes.

	Activity of CPT-I			Rates of fatty acid oxidation		
Additions	PP ($n = 10$)	PV ($n = 12$)	PP/PV	PP ($n = 10$)	PV ($n = 11$)	PP/PV
None	7.85±0.31	6.23±0.86[a]	1.26	38.22±5.16	28.66±2.53[a]	1.33
Change caused by						
Insulin (85 nM)	83±7	88±5	0.94	82±5	85±4	0.96
Glucagon (10 nM)	126±7	123±9	1.02	133±3	135±10	0.99
PMA (1 μM)	61±14	64±11	0.95	63±4	56±9	1.13
Ethanol (20 mM)	68±3	70±7	0.97	64±5	62±7	1.03
Acetaldehyde (5 mM)	74±9	67±5	1.10	62±10	69±8	0.90

periportal and perivenous hepatocytes. CPT-I activity was higher in periportal than in perivenous hepatocytes, as measured in the permeabilized-cell system (Table 5). Comparable results were obtained by Tosh *et al.* (1988) and by us (results not shown) with isolated mitochondria. We are aware that there is a too large difference between the activity of CPT-I, as measured in the permeabilized-cell assay, and the rates of fatty acid oxidation in isolated hepatocytes (see Table 5). Although it is difficult to know the actual reasons for this discrepancy, at least two factors should be considered. (i) In order to measure enzyme activity we permeabilize the plasma membrane and this causes the cytosol to leak out of the cell, leading to dilution of cytosolic components including malonyl-CoA. Thus the inhibition *in situ* by intracellular malonyl-CoA exerted on the enzyme in isolated hepatocytes does not take place when cells are permeabilized by digitonin. (ii) Enzyme activity is measured under optimal conditions in relation to supply of exogenous substrates (i.e. L-carnitine and long-chain acyl-CoA) as well as cofactors (e.g. KCl). Nevertheless, rates of fatty acid oxidation are determined in the presence of 0.4 mM fatty acid. This concentration, although near to physiological, allows rates of fatty acid oxidation which are quite far from maximal (results not shown).

Inhibition of CPT-I activity by physiological (i.e. micromolar) concentrations of malonyl-CoA is a complex but well-documented property of the enzyme (Zammit, 1986). Furthermore, long-term variations of CPT-I activity are usually accompanied by parallel changes in enzyme sensitivity to malonyl-CoA (Bremer, 1981; Stakkestad & Bremer, 1983; Saggerson & Carpenter, 1986; Cook & Gamble, 1987; Guzmán *et al.*, 1987). Therefore we isolated mitochondria from the two cell sub-populations and determined the IC_{50} (concentration of inhibitor causing 50% of maximal inhibition) of the enzyme for malonyl-CoA. The IC_{50} value was 6.33 ± 0.84 μM in periportal hepatocytes and 4.42 ± 0.51 μM in perivenous cells ($P < 0.01$ by the paired t test). In addition, perivenous cells were more sensitive than periportal hepatocytes to exogenously added malonyl-CoA in the permeabilized-cell system (results not shown). Hence the increased capacity of periportal hepatocytes to oxidize fatty acids might be accounted for by a higher activity of CPT-I and a lower sensitivity of this enzyme to inhibition by malonyl-CoA. Nevertheless, it must be taken into account that ketogenesis from octanoate (which can enter the mitochondria independently of CPT-I) has also been observed to be higher in periportal than in perivenous cells (Tosh *et al.*, 1988).

In order to investigate whether the above-mentioned variations in the capacity of fatty acid oxidation and in the activity of CPT-I were accompanied by alterations in their regulatory properties, we studied the short-term response of these two parameters to cellular effectors. The use of a permeabilized-hepatocyte system is essential to preserve the short-term alterations induced on the CPT-I enzyme by the distinct cellular modulators studied herein (Guzmán & Geelen, 1988*b*,*c*). In agreement with previous results, both the rate of fatty acid oxidation and the activity of CPT-I were increased by glucagon and decreased by insulin, PMA, ethanol and acetaldehyde (Guzmán & Geelen, 1988*b*,*c*). However, the quantitative effects exerted by all these compounds when added to the cell incubation medium were not significantly different in periportal and perivenous hepatocytes (Table 5), supporting the view outlined above that the short-term modulation of fatty acid metabolism does not differ between the two cell sub-populations.

Conclusions

Hepatocytes isolated from the periportal and the perivenous zone of the liver acinus constitute a very useful tool for the study of hepatic metabolic zonation. Together with results published by other authors (Chen & Katz, 1988, and references cited therein), a general model may be inferred in which the perivenous zone of the liver seems essentially to perform lipogenic processes, whereas gluconeogenesis, glycogen synthesis and (not so markedly) fatty acid oxidation take place preferentially in the periportal area of this organ. In addition, no differences between periportal and perivenous hepatocytes seem to exist in relation to the short-term modulation by cellular effectors of lipid (Tosh *et al.*, 1988; the present work) as well as carbohydrate metabolism (Keppens & De Wulf, 1988; Tosh *et al.*, 1988). However, it has been shown that glucagon causes greater long-term stimulation of gluconeogenesis and ketogenesis in periportal than in perivenous hepatocytes, and this has been related to the higher density of rough endoplasmic reticulum in the periportal as compared with the perivenous zone of the liver (Tosh *et al.*, 1988).

From all these investigations it is evident that studies on hepatic metabolism should be carried out separately in the two different hepatocyte sub-populations, instead of being performed with the classical heterogeneous cell preparations obtained from the whole liver. Nevertheless, the situation *in vivo* may be even more complicated, owing to the existence of concentration gradients of nutrients, hormones and oxygen (Jungermann, 1986; Gumucio, 1989).

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