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Periportal zonation of the cytosolic acetyl-CoA synthetase of male rat liver

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Several important metabolic functions of the mammalian liver have been shown to be located in zones with respect to the complex microcirculation of the organ. The zonal distribution of the cytosolic component of the acetyl-CoA synthetase activity has been investigated using the dual-digitonin-pulse-perfusion technique, which allows highly zone-selective sampling of cytosol from the periportal and perivenous zone of rat liver. Approximately 80% of the cytosolic enzymes are eluted from the hepatocytes in the periportal and perivenous sub-zones affected by digitonin, while less than 1% of the glutamate dehydrogenase activity (a marker enzyme of the mitochondrial compartment) is eluted. A twofold higher activity of the cytosolic form of acetyl-CoA synthetase is found in the periportal zone compared to the perivenous zone in fed male rats. Following a fasting/refeeding transition, this activity gradient is abolished in a manner similar to that observed for the enzyme acetyl-CoA carboxylase. Since the latter enzyme is utilizing the product of acetyl-CoA synthetase, acetyl-CoA, the similarity in the observed regulation suggests a functional coupling between cytosolic acetate activation and fatty-acid synthesis.

The enzyme acetyl-CoA synthetase (CoASAc synthetase) is expressed in the mammalian liver where it serves the function of activating acetate which is either supplied to the liver or produced metabolically, e.g. during ethanol metabolism [1]. The CoASAc synthetase is present both in the cytosolic and the mitochondrial compartments, but as two different proteins [2–5]. The total capacity of acetate uptake in the liver depends upon the acetate concentration. At 10 mM, it was reported to be 1 μmol·(g wet mass)⁻¹·min⁻¹ [5].

In recent years, it has been demonstrated that several metabolic pathways display functional zonation in the liver. Thus, the processes of gluconeogenesis and urea synthesis are primarily located in the periportal zone, while most of the cytochrome-P450-catalyzed reactions are primarily located in the perivenous zone (for review, see [6 and 7]). A few enzymes, glutamine synthetase and the ethanol-inducible cytochrome P450 IIE1 are exclusively located around the central vein of the liver lobule [8, 9]. Similarly, the enzyme hydroxymethylglutaryl-CoA reductase appear to be exclusively located in the periportal zone [10].

While microdissection studies have shown a preferential perivenous zonation of acetyl-CoA carboxylase (CoASAc carboxylase) [11], a key enzyme regulating fatty-acid synthesis, investigations applying the dual digitonin-perfusion technique has demonstrated that CoASAc carboxylase activity in the fed and fasted male rat is significantly higher in the periportal zone, caused almost entirely by a higher specific activity of the enzyme in that zone [12, 13]. Since acetate is a substrate for lipogenesis, we were therefore interested in whether CoASAc synthetase showed any zonation. In addition, since the pathological changes of long-term ethanol abuse is primarily seen in the perivenous zone [14], it is of some interest to elucidate the zonation of the enzymes involved in ethanol metabolism. Data obtained by microdissection and microchemical analysis show that both alcohol dehydrogenase and aldehyde dehydrogenase are almost evenly distributed in the liver parenchyma of the male rat [15, 16], and since only a fraction (0–45%) of the acetate formed by the liver during ethanol oxidation is actually being metabolized in the liver [17], one might speculate that a contributing factor could be a significant periportal zonation of CoASAc synthetase.

In the present investigation, we find 1.5–2-fold higher activity of cytosolic CoASAc synthetase in the periportal zone of the male rat liver compared with the perivenous zone in the fed state, but not in the fasted/refed state. These results have been presented in a preliminary form [18].

METHODS

Male Wistar rats, 200—220 g, were used. Three nutritional states were studied: well-fed, 48-h starved and re-fed animals (48-h starved and 48-h re-fed). Rats were fed ordinary rat chow except during re-feeding where high carbohydrate chow was given.

Prior to digitonin-pulse perfusion [19], the small, lower-right liver lobe was removed and homogenized in Krebs-
Henseleit buffer with 20 mM Hepes, pH 7.4 but without Ca^{2+} for measurements of enzyme activities. The eluates obtained after digitonin treatment were collected in fractions of 2 ml and rapidly cooled on ice, adding dithiothreitol to a final concentration of 4 mM. The activity of alanine aminotransferase, pyruvate kinase and glutamate dehydrogenase was measured by standard assays [20] and glutamine synthetase as described in [21]. The protein concentration in the eluates was measured according to [22]. CoASAc synthetase activity was measured by a modification of the method described in [23]. In brief, the following procedure was used, based on the two reactions

\[ ^{14}\text{C]acetate} + \text{CoA} + \text{ATP} \rightarrow ^{14}\text{C]acetyl-CoA} + \text{PPi} + \text{AMP} \]

\[ ^{14}\text{C]acetyl-CoA} + \text{oxaloacetate} \rightarrow ^{14}\text{C]citrate} + \text{CoA}. \]

25–50 μl of each eluate fraction was incubated for 15 min at 37°C in 50 mM KH_2PO_4, 100 mM KCl, 8 mM MgCl_2, pH 7.3, with 10 mM sodium acetate, 500000 dpm/assay [2-^{14}\text{C]sodium acetate, 0.4 mM CoA, 8 mM ATP, 10 mM oxaloacetate, 2 mM dithiothreitol and 3 U/assay citrate synthase] in a total volume of 0.5 ml. The reactions were terminated by additions of 50 μl 2.5 M HCl. The unreacted acetic acid was removed by freeze-drying and the radioactivity of the non-volatile citrate was counted.

The enzyme activities in the eluates are expressed as U/mg total eluted protein. Since it can be assumed that the protein concentration is equal in the periportal and the perivenous zone [24], zonation may be quantitated by comparing periportal and perivenous activities. Note that the activity in the biopsies is expressed/mg total protein, while in the eluate, activity is expressed/mg eluate protein (cytosolic protein), which is approximately 30% of the total protein [24].

RESULTS

The dual-digitonin-perfusion technique samples cytosol from the periportal and the perivenous zone of the same liver at a very high zonal selectivity, as has been demonstrated recently in histological studies [25, 26]. Under the conditions applied, the periportal and the perivenous samples represent approximately 15% each of the total liver parenchyma [25]. The enzyme activities of some marker enzymes are given in Table 1, which demonstrates the highly significant 4–10-fold periportal/perivenous activity gradient of alanine aminotransferase, depending upon the nutritional state, as well as a more than 100-fold periportal-perivenous activity gradient of glutamine synthetase [9]. Pyruvate kinase shows no significant zonation [12]. Since a biopsy has been removed from each liver prior to the digitonin-pulse-treatment, it is possible to compare the enzyme activities of the eluate with the activities measured in the biopsy representing the whole liver. For pyruvate kinase a 2–3-fold higher specific activity is found in the eluate compared with the homogenate of the biopsy. Since pyruvate kinase is essentially unzonated, this suggests that only the cytosolic space is being sampled, a conclusion which is confirmed by the fact that less than 0.2% of the activity of the mitochondrial enzyme glutamate dehydrogenase is observed in the eluates (see Table 1).

The activity of the CoASAc synthetase in the periportal and perivenous eluates in the three nutritional states is shown in Fig. 1. In the fed state, the CoASAc synthetase activity is zonated approximately 1.7-fold compared to the periportal zone. Exposing the animals to a fasting/re-feeding transition essentially abolishes this zonation, because of a selective increase in the activity in the perivenous zone. In the 48-h fasted rat, the activity of CoASAc synthetase is less than half the value found in the fed state; however, the periportal/perivenous zonation is still present. Due to the low amount of enzyme in the perivenous eluates from fasted rats, the activity could, however, not be accurately determined (activity < 4 μU/mg eluted protein, Fig. 1). We have attempted to evaluate the shape of the CoASAc synthetase activity gradient through the liver sinusoid by multiple digitonin-pulse-perfusion as described in [27]. This method applies consecutive digitonin pulses whereby cytosol from different zones of the lobule is sampled. The result of these measurements (not shown) suggests a linear periportal/perivenous gradient, as was indeed found for CoASAc carboxylase [27].

DISCUSSION

The present study of the zonation of CoASAc synthetase in the rat male liver was undertaken for the following reasons:

a) Although subject to unresolved controversy [11, 28, 29], it has been shown recently that the rate-limiting enzyme for fatty-acid synthesis, CoASAc carboxylase, has a higher activity in the periportal zone in the fed, male rat liver. Interestingly, this activity gradient could be almost entirely accounted for by a difference in the specific activity of the CoASAc carboxylase protein isolated from the two zones. Furthermore, the nutritional transition caused by fasting and re-feeding almost abolished the specific-activity gradient [13]. This effect can probably be explained by a more pronounced increase in the activity of CoASAc carboxylase phosphatase in the perivenous zone [13]. Since acetate is a substrate for lipogenesis, it is interesting to note that the CoASAc synthetase activity gradient, as demonstrated in the present paper, responds to fasting/re-feeding as does CoASAc carboxylase. In this context, it should be noted that with the dual-digitonin-perfusion technique as applied here, only the cytosolic component of CoASAc synthetase was studied, as indicated by the fact that < 0.2% of the mitochondrial marker enzyme glutamate dehydrogenase was found in the eluate (Table 1). Thus, the data of Fig. 1 refer to cytosolic CoASAc synthetase only, which comprises about 80% of the total CoASAc synthetase activity in the fed state and about 60% in the fasted state [4, 5]. It has been reported that the mitochondrial CoASAc synthetase activity does not respond to changes of nutritional state [4, 5].

b) The other reason for the present study of CoASAc synthetase zonation is related to the fact that only a minor fraction of the acetate formed by the liver during ethanol oxidation is being taken up by the liver itself [17]. Since both alcohol dehydrogenase and aldehyde dehydrogenase display little or no zonation in the male rat liver [15, 16], one explanation for the low acetate uptake might be a significant zonation of CoASAc synthetase in the liver lobule; hence a complete periportal location of CoASAc synthetase would prevent most of the acetate formed by ethanol oxidation from being taken up by the liver. However, the moderate zonation of CoASAc synthetase observed in the present study and the fact that the zonation of this enzyme activity was abolished in the fasted/refed state, speaks against such a hypothesis as far as the cytosolic CoASAc synthetase is concerned. Furthermore, recent results (Knudsen, Quistorff and Grunnet, unpublished data) suggest that the inhibition of acetate uptake by ethanol in the liver is due to inhibition of the tricarboxylic acid cycle, while ketone-body formation is unchanged, the effect being a higher apparent K_m value for acetate uptake.
Table 1. Activity of marker enzymes of rat liver and effects of the nutritional state. Samples of the cytosolic compartment of perportal and perivenous cells of male rat liver were obtained by the digitonin-pulse-perfusion technique [12, 24]. Animals were either fed, fasted (48 h) or fasted/re-fed (48 h/48 h) prior to the experiment. Mean ± SD with n = 4 in all three groups. Note that the biopsy data before and after digitonin perfusion are expressed/mg total protein, while the activity measured in the eluates are expressed/mg total eluted protein (cytosolic protein). ALAT, alanine aminotransferase; GIDH, glutamate dehydrogenase; GS, glutamine synthase; PK, pyruvate kinase.

<table>
<thead>
<tr>
<th>Nutritional state</th>
<th>Marker enzyme</th>
<th>Biopsy sample before digitonin perfusion</th>
<th>Biopsy sample after digitonin perfusion</th>
<th>Cytosolic eluate sample perportal sample</th>
<th>Cytosolic eluate sample perivenous sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmol · min⁻¹ · (mg total protein)⁻¹</td>
<td>nmol · min⁻¹ · (mg total protein)⁻¹</td>
<td>nmol · min⁻¹ · (mg total eluted protein)⁻¹</td>
<td>nmol · min⁻¹ · (mg total eluted protein)⁻¹</td>
</tr>
<tr>
<td>Fed</td>
<td>PK</td>
<td>498 ± 99</td>
<td>484 ± 64</td>
<td>979 ± 138</td>
<td>1011 ± 62</td>
</tr>
<tr>
<td></td>
<td>ALAT</td>
<td>101 ± 16</td>
<td>79 ± 6</td>
<td>426 ± 126</td>
<td>44.5 ± 26</td>
</tr>
<tr>
<td></td>
<td>GS</td>
<td>31.2 ± 3.5</td>
<td>5.9 ± 1.3</td>
<td>4.2 ± 5.8</td>
<td>64.6 ± 90</td>
</tr>
<tr>
<td></td>
<td>GIDH</td>
<td>1021 ± 118</td>
<td>1093 ± 117</td>
<td>2.7 ± 1.5</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>Fasted</td>
<td>PK</td>
<td>200 ± 34</td>
<td>201 ± 72</td>
<td>495 ± 79</td>
<td>518 ± 92</td>
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<tr>
<td></td>
<td>ALAT</td>
<td>170 ± 17</td>
<td>162 ± 33</td>
<td>596 ± 170</td>
<td>116 ± 69</td>
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<tr>
<td></td>
<td>GS</td>
<td>21.1 ± 3.6</td>
<td>3.7 ± 1.4</td>
<td>5.4 ± 51</td>
<td>443 ± 54</td>
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<tr>
<td></td>
<td>GIDH</td>
<td>1561 ± 71</td>
<td>1899 ± 366</td>
<td>2.9 ± 2.4</td>
<td>2.3 ± 0.3</td>
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<tr>
<td>Fasted/re-fed</td>
<td>PK</td>
<td>722 ± 86</td>
<td>588 ± 9</td>
<td>1516 ± 232</td>
<td>1444 ± 130</td>
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<tr>
<td></td>
<td>ALAT</td>
<td>131 ± 27</td>
<td>113 ± 264</td>
<td>338 ± 42</td>
<td>94 ± 17</td>
</tr>
<tr>
<td></td>
<td>GS</td>
<td>15.3 ± 2.8</td>
<td>2.9 ± 0.8</td>
<td>2.6 ± 1.1</td>
<td>279 ± 46</td>
</tr>
<tr>
<td></td>
<td>GIDH</td>
<td>973 ± 97</td>
<td>1149 ± 65</td>
<td>6.5 ± 2.8</td>
<td>4.0 ± 0.9</td>
</tr>
</tbody>
</table>

1 Perivenous activities which are significantly different from perportal activities as evaluated by the paired-data t-test, p < 0.01.

Fig. 1. Zonation of acetyl-CoA synthetase and the effect of the nutritional state. Samples of cytosol from perportal and perivenous cells were obtained from male rat liver by the digitonin-perfusion technique. Animals were either fed, fasted (48 h) or fasted/re-fed (48 h/48 h). Hatched bars represent perportal and open bars perivenous samples, respectively. Enzyme activity is expressed as µU/mg total eluted protein. Mean ± SD with n = 11, 3 and 9 in the fed, fasted and fasted/re-fed groups, respectively. *, p < 0.05 as evaluated by paired-data t-test.

All in all, the present paper suggests that the cytosolic CoA-SAc synthetase activity may be functionally coupled to fatty-acid synthesis, although its quantitative role may be small compared with the mitochondrial CoA-SAc synthetase [30].

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REFERENCES