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Distribution and induction of cytochrome P-450 and two cytochrome P-450-dependent monooxygenase activities in rat liver parenchymal cell subpopulations separated by centrifugal elutriation

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Abstract. Liver parenchymal cells from the periportal and centrilobular zones differ in their morphological, biochemical and functional characteristics. In an effort to obtain fractions enriched in either periportal or centrilobular cells, isolated rat liver parenchymal cells were separated into five subpopulations by centrifugal elutriation. The mean diameters of the cells present in fractions I–V were 19.6, 21.1, 21.8, 22.7 and 23.5 μm, respectively. The content of cytochrome P-450 as well as benzphetamine N-demethylase and 7-ethoxyresorufin O-deethylase activities were higher in the larger parenchymal cells than in the smaller ones. After administration of phenobarbital the content of cytochrome P-450 was approximately two-fold greater in the cells present in fractions 3–5, when compared to the same subpopulations isolated from untreated rats; the activity of benzphetamine N-demethylase was enhanced to a similar extent in all five fractions. 3-Methylcholanthrene treatment resulted in a significant increase of cytochrome P-450 content and 7-ethoxyresorufin O-deethylase activity in all five fractions: both parameters were slightly higher in fractions 4 and 5 than in fractions 1 and 2. In conclusion, the elutriated liver parenchymal cells seem to preserve the biochemical heterogeneity observed in the intact liver; the potential enrichment of periportal and centrilobular cells in the different fractions by centrifugal elutriation is discussed.

Key words: Liver parenchymal cells – cytochrome P-450 – Centrifugal elutriation – 3-Methylcholanthrene – Phenobarbital

Introduction

Parenchymal cells within the hepatic lobules of the rat are morphologically and biochemically heterogeneous (Novikoff 1959; Shank et al. 1959; Loud 1968; Jungermann and Katz 1982). It has been previously shown that the concentration of cytochrome P-450 and the activities of most cytochrome P-450-dependent monooxygenases are relatively higher in the centrilobular than in the periportal regions of the rat liver (Baron et al. 1978; Gooding et al. 1978; Baron and Kawabata 1983); further, these studies revealed that pretreatment of the animals with phenobarbital intensified this gradation across the liver lobule, whereas after administration of 3-methylcholanthrene the concentration of cytochrome P-450 and the monooxygenase activities were more uniformly distributed. Since very reactive and toxic metabolites are often formed during cytochrome P-450-catalyzed monooxygenations of hepatotoxins, the intralobular distribution of different cytochromes P-450 may be a crucial factor in determining the location of the damage within the hepatic lobules after exposure to several hepatotoxic compounds.

A method to isolate parenchymal cells originating in periportal or centrilobular regions of the liver would provide an extremely valuable tool for the study of the mechanism(s) underlying the toxic effects of many chemicals which only affect a particular cell subpopulation within the liver acinus. Recently, centrifugal elutriation has been used to separate isolated rat hepatocytes into several fractions, which contain cells of different sizes (Sumner et al. 1983; Klinger et al. 1985; Willson et al. 1985; Gumucio et al. 1986). In order to analyze the utility of centrifugal elutriation in obtaining fractions enriched in either periportal or centrilobular cells, liver parenchymal cells isolated from untreated and phenobarbital- or 3-methylcholanthrene-pretreated rats were separated into five subpopulations by centrifugal elutriation; the criteria used to assess the possible acinar origin of the separated subpopulations were: a) their activities of glutamate pyruvate transaminase and pyruvate kinase; b) their content of cytochrome P-450; c) their activities of two cytochrome P-450-dependent monooxygenases, 7-ethoxyresorufin O-deethylase and benzphetamine N-demethylase. The potential "enrichment" of periportal and centrilobular cells in the different fractions by centrifugal elutriation is discussed.

Materials and methods

Chemicals. Collagenase was purchased from Boehringer (Mannheim, FRG); phenobarbital from Merck (Darmstadt, FRG); 3-methylcholanthrene from Aldrich (Steinheim, FRG); 7-ethoxyresorufin from Pierce (Rodgau, FRG); and benzphetamine from Upjohn (Kalamazoo, USA). All other chemicals used were of the highest purity available.

Animals and pretreatments. Male Sprague-Dawley rats (300–350 g body weight) from the Süddeutsche Versuchstierfarm (Tuttlingen, FRG) were housed in plastic cages on a fixed day and night cycle and fed a standard rat chow

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until used. The animals were pretreated i.p. with phenobarbital (100 mg/kg body weight/day) in 0.85% saline or 3-methylcholanthrene (25 mg/kg body weight/day) for 3 consecutive days. Untreated rats received appropriate volumes of corn oil. Liver parenchymal cells were isolated 24 h after the last injection.

Isolation of liver parenchymal cells. Total liver cell suspensions were obtained using a collagenase perfusion method (Glatt et al. 1981). The perfusion medium used for all steps of the cell isolation procedure was the Krebs-Henseleit buffer, containing 25 mM 4-(2-hydroxymethyl)-1-piperazineethanesulfonic acid (pH 7.4), 0.5% D-glucose, insulin (8 mg/l), and the mixture of amino acids recommended by Seglen (1976). Briefly, the liver was perfused for 5 min through the portal vein in a non-recirculatory manner with Ca^{2+}-free Krebs-Henseleit buffer. This was followed by a recirculatory perfusion of 10 min with 0.5 mM ethylene glycol bis (β-aminoethyl ether)N,N,N',N'-tetraacetic acid in the above-mentioned buffer to disrupt the desmosomes. The perfusion medium was then changed to Krebs-Henseleit buffer containing 2.5 mM $CaCl_2$ and 0.05% collagenase and the perfusion continued for 35–45 min. Finally, the Glisson's capsule was removed from the liver and the cells filtered through gauze to remove clumps. The suspension was centrifuged and washed three times at 50 *g* for 3 min, and the supernatants discarded. The final pellet contained >95% parenchymal cells.

Fraction of isolated liver parenchymal cells. The parenchymal cells in the pellet were resuspended and filtered through a 100 micron and then a 60 micron nylon mesh. Elutriation was performed as described by Bernaert et al. (1979). A JE-5B elutriator rotor with a standard separation chamber (Beckman Instruments, Palo Alto, California, USA) was used in a J-6M/E Beckman centrifuge at a speed of 840 rpm. The rotor, the elutriation medium (Krebs-Henseleit buffer) and the samples collected during the separation were kept at 4° C to preserve the integrity of the cells. The parenchymal cell suspension ($5-10 \times 10^7$ cells) was loaded into the elutriation system with an initial flow rate of 15 ml/min. Parenchymal cell fractions I–V were obtained by using flow rates of 20, 25, 30, 35 and 45 ml/min, respectively; for each fraction 150 ml of eluate were collected. The viability of each preparation was assessed by determining the proportion of cells that excluded 0.4% trypan blue. Morphometric data of the elutriated subpopulations were obtained by means of a ZM Coulter counter coupled to a C-1000 channelyzer (Coulter Electronics GmbH, Krefeld, FRG).

Assays. All determinations were performed on the 10000 *g* supernatants of the sonicated cell subpopulations. Cytochrome P-450 content was measured as described by Omura and Sato (1964). Benzphetamine N-demethylase activity was determined colorimetrically by following the formation of formaldehyde (Lu et al. 1972). 7-Ethoxyresorufin O-deethylase activity was measured fluorimetrically according to the method of Burke and Mayer (1974). Glutamate pyruvate transaminase activity was determined colorimetrically as described by Bergmeyer and Bernt (1974), while pyruvate kinase activity was measured according to van Berkel et al. (1970). Proteins were measured by the method of Lowry et al. (1951) with bovine serum albumin as standard.

Statistics. Statistical analyses of the results were carried out using Dunnett's test for multiple comparisons with a control (Dunnett 1964).

Results

Liver parenchymal cells from untreated and phenobarbital- or 3-methylcholanthrene-pretreated rats were isolated by a collagenase perfusion method and further separated into five subpopulations by centrifugal elutriation. The cell recovery following elutriation was of about 90% and the number of cells in the five fractions was similar for the three experimental groups. The viability of the cell suspensions before loading the elutriation chamber amounted to 88±4% (n = 18), whereas the percentage of cells excluding trypan blue in the elutriated subpopulations from untreated and induced animals averaged 95%. Thus, a high number of non-viable cells was removed during the elutriation process.

The mean diameters (expressed as means ±S.D.) of the parenchymal cells from untreated rats (n = 8) present in fractions 1–5 were 19.6±0.3, 21.1±0.5, 21.8±0.6, 22.7±0.3 and 23.5±0.4 μm, respectively, while the subpopulations from phenobarbital-pretreated rats (n = 5) had mean cell diameters of 20.3±0.5, 21.7±0.7, 23.1±0.7, 24.0±0.4 and 25.6±0.5 μm. The elutriated parenchymal cells from 3-methylcholanthrene-pretreated rats (n = 5) present in fractions 1–5 had mean diameters of 21.8±0.6, 22.7±0.6, 23.4±0.3, 23.9±0.5 and 24.4±0.4 μm, respectively.

The activities of glutamate pyruvate transaminase and pyruvate kinase in the five fractions obtained from untreated rats are shown in Fig. 1. Although not statistically significant, glutamate pyruvate transaminase activities in fractions 1–3 were 1.4-fold higher than that in fraction 5, while pyruvate kinase activities in fractions 3–5 were approximately 1.3-fold higher than those of fractions 1 and 2.

The cytochrome P-450 content in the five subpopulations from the three experimental groups are shown in Fig. 2. In untreated animals the cellular concentration of cytochrome P-450 increased from fractions 1 to 5, the

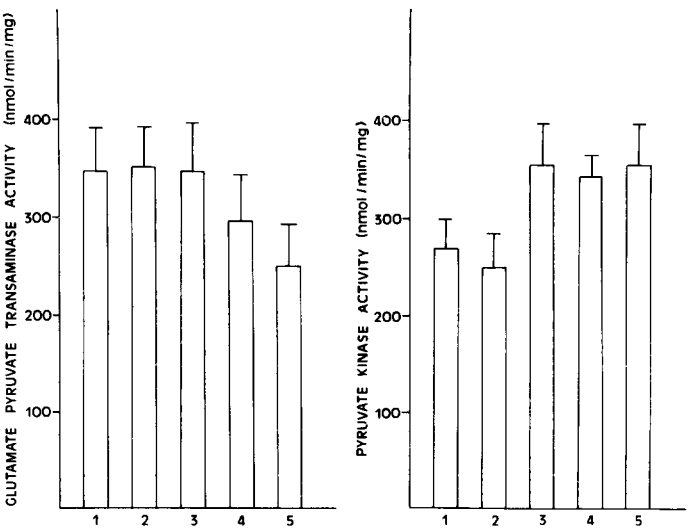

Fig. 1. Glutamate pyruvate transaminase and pyruvate kinase activities in the elutriated parenchymal cell fractions I–V from untreated rats (n = 4). Values are expressed as means ±SD

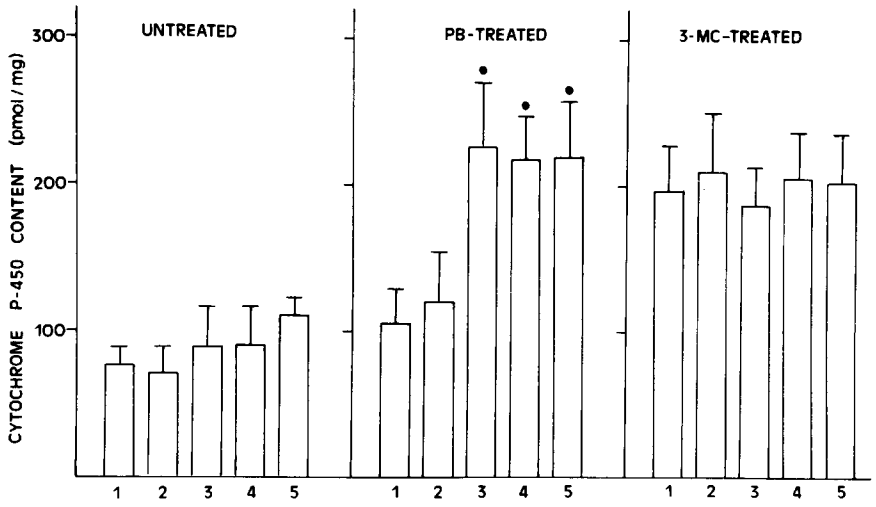

Fig. 2. Cytochrome P-450 content in the elutriated parenchymal cell fractions 1–5 from untreated and induced rats. Values are expressed as means ±SD of four experiments per group. Indicates significantly different from the corresponding fraction 1 ($p < 0.05$, Dunnett's test)

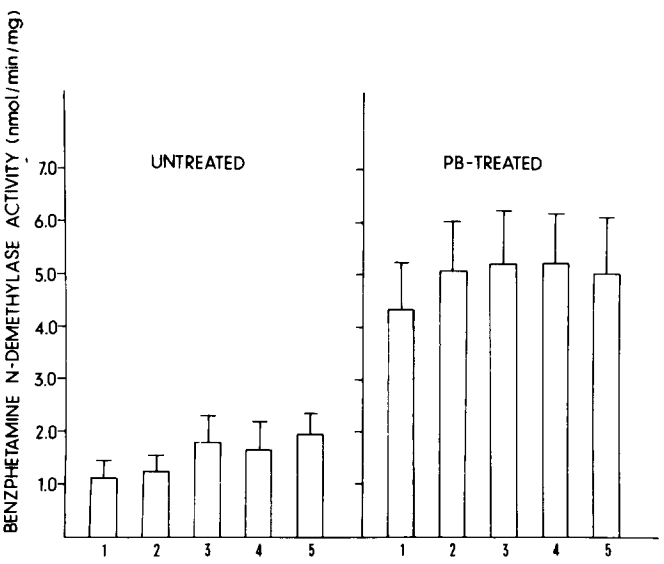

Fig. 3. Benzphetamine N-demethylase activity in the elutriated parenchymal cell fractions 1–5 from untreated and phenobarbital-pretreated rats. Values are expressed as means ±SD of five experiments per group

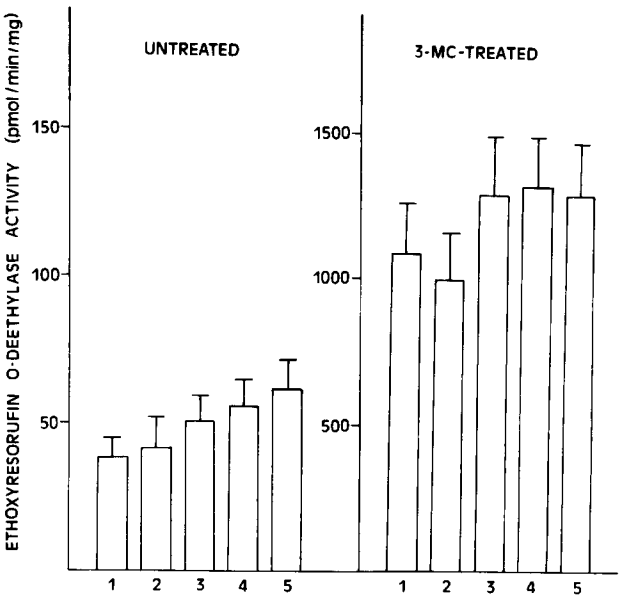

Fig. 4. Ethoxyresorufin O-deethylase activity in the elutriated parenchymal cell fractions 1–5 from untreated and 3-methylcholanthrene-pretreated rats. Values are expressed as means ±SD of five experiments per group

amount of the hemeprotein being approximately 1.4-fold higher in fraction 5 than in fraction 1 (statistically not significant). After administration of phenobarbital the content of cytochrome P-450 increased by a factor of two in the cells present in fractions 3–5 when compared to the same subpopulations isolated from untreated rats, whereas the amount of cytochrome P-450 in fractions 1 and 2 did not vary significantly. Pretreatment of the animals with 3-methylcholanthrene led to a greater increase of cytochrome P-450 content in fractions 1 and 2 than in fractions 3–5, so that all five subpopulations had a similar concentration of cytochrome P-450.

The N-demethylation of benzphetamine is preferentially catalyzed by those isoenzymes of cytochrome P-450 that are inducible by phenobarbital (Lu et al. 1972). The activity of benzphetamine N-demethylase in the five subpopulations from untreated and phenobarbital-pretreated rats is shown in Fig. 3. In the case of untreated rats, benzphetamine N-demethylase activity increased from fraction 1 to fraction 5 by a factor of two. After administration of phenobarbital, benzphetamine N-demethylase activity in all five subpopulations was strongly induced.

The O-deethylation of 7-ethoxyresorufin is catalyzed by the 3-methylcholanthrene-inducible isoenzymes of cytochrome P-450 (Burke and Mayer 1974). 7-Ethoxyresorufin O-deethylase activity within the elutriated subpopulations from untreated and 3-methylcholanthrene-pretreated rats is shown in Fig. 4. In untreated animals the deethylating activity increased from fraction 1 to fraction 5 by a factor of 1.7. Pretreatment with 3-methylcholanthrene led to a 28- and 20-fold induction of 7-ethoxyresorufin O-deethylase activity in fractions 1 and 5, respectively.

Discussion

The observed differences in cell size among the five parenchymal cell subpopulations separated by centrifugal elutriation are in accordance with previous reports (Bernaert et al. 1979; Wanson et al. 1980; Klinger et al. 1985; Willson et al. 1985; Gumucio et al. 1986). Morphometric studies from the intact liver (Schmucker et al. 1978) showed that centrilobular cells were consistently larger than periportal cells. Furthermore, Wanson et al. (1975) reported that administration of phenobarbital to rats preferentially increased the size of centrilobular cells; interestingly, in the present study pretreatment of the animals with phenobarbital led to a significantly greater increase of cell size in fractions 3–5 (i.e. those containing the larger cells in the case of untreated rats) than in fractions 1 and 2.

The fact that the cytochrome P-450 content was higher in the large than in the small cells from untreated rats (fractions 5/fraction 1 ratio = 1.4) is in agreement with previous studies on parenchymal cell subpopulations isolated by centrifugal elutriation (Willson et al. 1985), density gradient (Tonda et al. 1983) and sedimentation velocity (Sweeney et al. 1978). In phenobarbital-pretreated animals cytochrome P-450 content rose 2-fold in fractions 3–5, while it remained unchanged in fractions 1 and 2; after administration of 3-methylcholanthrene, all five fractions had a similarly elevated cytochrome P-450 content. Taken together, these data suggest that fractions 1 and 2 might be enriched in parenchymal cells originating in periportal regions of the liver acini, while fractions 3–5 might contain parenchymal cells from the centrilobular regions of the liver acini. This suggestion is based on the following observations made on liver sections by means of immunohistochemical methods: a) cells lying within the centrilobular regions of the liver acini from untreated rats contain greater concentrations of the cytochrome P-450 forms induced by phenobarbital and 3-methylcholanthrene than do cells lying within the periportal regions (Gooding et al. 1978; Baron et al. 1981; Ohnishi et al. 1982; Wolf et al. 1984); b) after treatment with phenobarbital, cytochrome P-450 is primarily induced within centrilobular cells (Gooding et al. 1978; Smith et al. 1981; Ohnishi et al. 1982; Wolf et al. 1984); c) the administration of 3-methylcholanthrene to rats results in similar degrees of induction of cytochrome P-450 within midzonal and periportal cells, but significantly less induction is detected within centrilobular cells, the concentrations of cytochrome P-450 within the three regions of the liver acini being therefore approximately equal (Baron et al. 1982).

In untreated animals, the distribution of benzphetamine N-demethylase and 7-ethoxyresorufin O-deethylase activities within the five cell fractions paralleled that of cytochrome P-450 content, the fraction 5/fraction 1 activity ratios being 1.7 for 7-ethoxyresorufin O-deethylase and 2.0 for benzphetamine N-demethylase. These data are in agreement with a recent study (Bengtsson et al. 1987) in which centrilobular parenchymal cells prepared by a digitonin-collagenase perfusion of the liver showed higher activities of 7-ethoxycoumarin O-deethylase, 7-ethoxyresurufin O-deethylase and benzo(*a*)pyrene hydroxylase than periportal cells, the differences in the three enzyme activities between the two subpopulations ranging from 1.5 to 2.0.

The induction of benzphetamine N-demethylase by phenobarbital was greater in fractions 1 and 2 than in fractions 3–5, so that only a 1.2-fold difference in benzphetamine N-demethylase activity between large and small cells was observed. Bengtsson et al. (1987) have previously shown that induction of several cytochrome P-450-dependent monooxygenase activities by phenobarbital occurs panacinarily (i.e. relatively more in the periportal regions of the liver acini), thus diminishing the original centrilobular dominance. In contrast to our data and the above-mentioned study, Tonda et al. (1983) reported a 2-fold and Willson et al. (1985) a 5-fold difference in 7-ethoxycoumarin O-deethylase activity between small and large parenchymal cells, phenobarbital therefore exaggerating the centrilobular dominance. Although benzphetamine N-demethylase activity after phenobarbital treatment was similar in all five fractions, the total cytochrome P-450 content increased by a factor of two in fractions 3–5, but not in fractions 1 and 2, when compared to the same subpopulations isolated from untreated rats. This may be due to the fact that the N-demethylation of benzphetamine is mainly catalyzed by cytochrome P-450IIB1 (Guengerich et al. 1982), only one of several cytochrome P-450 isoenzymes induced by phenobarbital. Indeed, an immunohistochemical study (Baron and Kawabata 1983) revealed that administration of phenobarbital to rats resulted in similar degrees of induction of cytochrome P-450IIB1 within midzonal and periportal parenchymal cells, but significantly less induction of this isoenzyme occured within centrilobular parenchymal cells.

7-Ethoxyresorufin O-deethylase activity was similar in large and small parenchymal cells isolated from 3-methylcholanthrene-pretreated rats, an observation also made by Tonda et al. (1983) in parenchymal cell subpopulations separated by density gradient centrifugation.

The possible acinar origin of the parenchymal cells present in the elutriated fractions was further assessed by measuring their glutamate pyruvate transaminase and pyruvate kinase activities: the former enzyme is two to three times more active in periportal parenchymal cells (Shank et al. 1959; Morrison et al. 1962; Welsh 1972), while the latter enzyme shows a preferential centrilobular location (Guder and Schmidt 1976). In the present study the fraction 5/fraction 1 activity ratios for glutamate pyruvate transaminase and pyruvate kinase were 0.7 and 1.3, respectively; similar differences have been observed among parenchymal cell subpopulations separated by density gradient centrifugation (Tonda et al. 1983; Vargas et al. 1987). However, the differences in these enzyme activities between the five fractions are not so marked as shown in the above-mentioned microdissection studies, thus suggesting that an overlap between the elutriated subpopulations does (at least in part) exist; the addition of DNAse I and/or albumin (up to 2%) to the elutriation buffer did not improve the separation (B. Seibert and P. Steinberg, unpublished observations).

In conclusion, the distribution of glutamate pyruvate transaminase and pyruvate kinase activities as well as the distribution and induction of cytochrome P-450 and two cytochrome P-450-dependent monooxygenase activities within the five cell subpopulations indicate that a partial separation of periportal (in fractions 1 and 2) and centrilobular parenchymal cells (in fractions 3–5) by centrifugal elutriation has been accomplished.

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