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CENTRILOBULAR EXPRESSION OF ETHANOL-INDUCIBLE CYTOCHROME P-450 (IIE1) IN RAT LIVER

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Western blot analysis of digitonin eluates as well as immunohistochemical analysis revealed a 30-fold higher concentration of cytochrome P-450IIE1 in the centrilobular than in the periportal regions of the rat liver. Ethanol treatment caused a selective centrilobular induction of P-450IIE1, whereas phenobarbital induced P-450IIB1/2 in both liver lobule regions. The heterogeneous distribution pattern of P-450IIE1 was also observed in cells isolated from either region and correlated to the relative content of P-450IIE1 mRNA in the two cell types. The regiospecific expression and induction of P-450IIE1 may explain why several hepatotoxins, known to be metabolized by this isozyme, primarily damage the centrilobular region in the liver.

The various components of the drug metabolizing system are not evenly distributed within the liver lobule (1). Epoxide hydrolase, conjugating enzymes and various forms of P-450 all have different relative concentrations in the periportal (zone 1), midzonal (zone 2) and centrilobular (zone 3) regions (2-4). Assessments of the lobular distribution of spectrally determinable P-450 as well as specific P-450 forms, have revealed an overall preferential centrilobular localization with about a 2-fold higher concentration in this region (4). Inducing agents have been found to affect the relative distribution of individual P-450 isozymes in nonuniform manners (3-5).

The regulation of the expression of the ethanol-inducible P-450IIE1 (P450j) isozyme appears to be of particular interest, since this enzyme specifically metabolizes a number of toxicologically important agents, such as acetaminophen (6), nitrosoamines (7), carbon tetrachloride (8) and various hydrocarbons like benzene (9) and pentane (10). We report here, by using immunohistochemistry and by immunoanalysis of cell samples obtained

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Abbrevations used:
P-450IIE1, ethanol inducible microsomal cytochrome P-450 (P-450j in rat liver); P-450IIB1/2, phenobarbital inducible microsomal cytochrome P-450 (P-450b/e in rat liver); ALT, alanine aminotransferase; GS, glutamine synthetase.

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preferentially from the periportal or centrilobular region by the digitonin-pulse technique (11-14), that P-450IIE1 appears to be expressed almost exclusively in the centrilobular region and that induction of the enzyme after chronic ethanol treatment is restricted to this region.

MATERIALS AND METHODS

Animals. Male rats of the Alko mixed strain (initial weight of 100-120 g) were provided with tap water and a standard R3 diet (Ewos AB, Södertälje, Sweden). Ethanol was administered chronically for four weeks by addition to the drinking fluid (15). The average daily ethanol intake during the whole period was 12.2 ± 2.2 g/kg body weight. The weight gain was similar to control animals receiving tap water and diet ad libitum. One control group of animals was treated for the last three days with phenobarbital (100 mg/kg body weight, daily) by stomach tube.

Materials. Anti-rat P-450IIE1 IgG was raised in rabbits as described (16). Anti-rat P-450IIB1/2 IgG was kindly donated by Dr B. Scholte.

Preparation of periportal and centrilobular hepatocytes and cell lysates. Hepatocytes from the periportal (pp) or centrilobular (cl) region were isolated by digitonin-collagenase perfusion (11, 12).

Preparation of periportal and centrilobular cell lysates by site directed digitonin infusion was performed as described before (13). Cell lysates from the periportal region was obtained by infusion of 7 mM digitonin via the portal vein of the perfused liver at a rate of 10 ml/min for 12-17 s. This was followed by collection of the lysate after immediate retrograde flushing. For collection of centrilobular lysates, digitonin was infused for 20-30 s via the hepatic veins and the eluate collected after antegrade flushing. This technique has recently been described in detail (14).

Alanine aminotransferase (ALT) activity was assayed as cited earlier (11) and glutamine synthetase (GS) was determined radioisotopically (17).

Assay of P-450 apoproteins and P-450IIE1 mRNA. Liver cells were homogenized (18) and 3 000 x g supernatant was prepared. The apo P-450 protein levels in the 3 000 x g supernatant or in digitonin eluates were analyzed by Western blot using 20 µg of protein in each well (cf. (18)). RNA was isolated and P-450IIE1 mRNA quantified by Northern blot as described elsewhere (18).

Immunohistochemistry. Liver tissue was fixed in buffered 4 % (v/v) formalin - 1 % (v/v) glutaraldehyde. The avidine-biotin-peroxidase complex method was performed for light microscopic immunocytochemistry. Deparaffinized sections of 5 µm thickness were treated with methanol containing 1 % (v/v) hydrogen peroxide and washed in PBS. The sections were incubated at 8°C for 12-14 hrs with rabbit antibodies (diluted 1:10-100) against P-450IIE1 using incubation chamber. After brief washing in PBS the slides were incubated for 30 min with biotin-labelled goat antirabbit immunoglobin followed by an avidin-biotin labelled peroxidase complex. Control reactions were performed without specific antiserum and with non-immune rabbit serum.

RESULTS

Digitonin eluates, obtained as described above from the centrilobular or the periportal region of livers from control, ethanol- or phenobarbital- treated rats, were analyzed with respect to their content of marker enzymes, that have an established heterogeneous lobular distribution, and with respect to their concentration of various forms of P-450, (Table I). The pronounced centrilobular localization of GS as well as the periportal dominance for ALT, are in full agreement with previous findings (11-14).

Immunodetectable cytochromes P-450IIB1/2 and IIE1 were analyzed in the eluates using Western blot and polyclonal antisera against the isozymes (Fig. 1 & Table I). The ethanol-inducible P-450IIE1 was almost exclusively present in centrilobular eluates. The amount of apo P-450IIE1 was here more than 30-fold higher than in lysates obtained from periportal regions. By contrast, P-450 IIC11 was much more evenly distributed with only

Table I. Levels of immunodetectable cytochromes P-450IIB1/2, IIE1 and activities of the marker enzymes alanine aminotransferase (ALT) and glutamine synthethase (GS) in cell lysates from periportal (pp) and centrilobular (cl) regions of the liver from variously treated rats. The P-450 values are given in relation to the amount of apo P-450 determined in eluates from the periportal region of control liver (100 %).

	Control		Ethanol		Phenobarbital	
	pp	cl	pp	cl	pp	cl
	n=7	n=7	n=7	n=7	n=3	n=3
P-450IIB1/2	< 30	100±84	< 30	125±115	3300±1000	7600±600
P-450IIE1	3±4	100±22	5±8	310±65		
ALT [1] /	1.12±0.23	0.30±0.12	0.85±0.17	0.21±0.09		
GS [2] /	0.82±0.50	580±95	0.70±0.18	540±160		

1/ µmol/mg protein, min
2/ nmol/mg protein, min

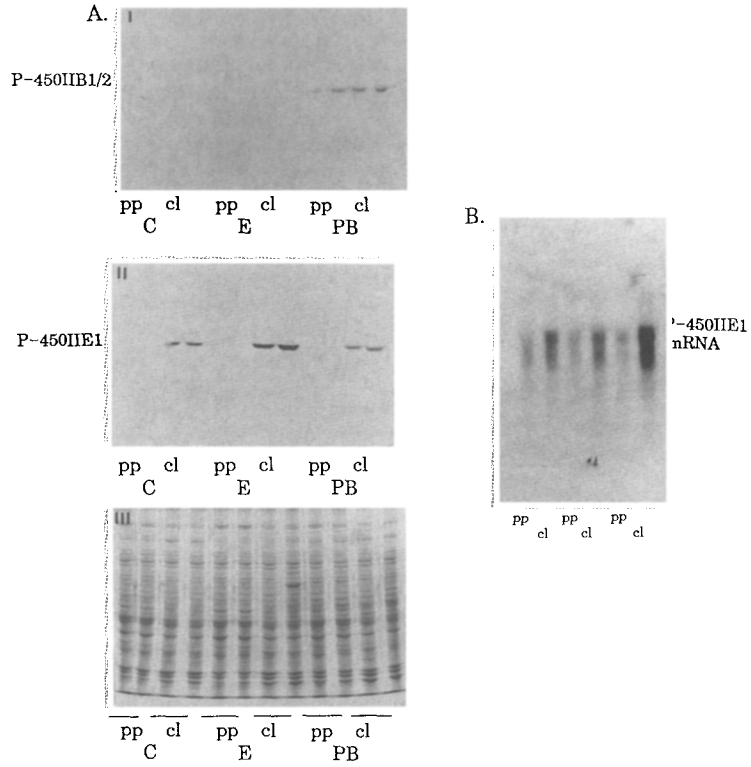

Fig. 1. A, SDS-electrophoresis of eluates from periportal (pp) and centrilobular regions (cl) of livers from control (C), ethanol (E) and phenobarbital (PB) treated rats. I & II, Western blot analysis of P-450IIB1/2 and IIE1, respectively; III, Coomassie staining. B, Northern blot analysis of P-450IIE1 mRNA in isolated control hepatocytes from periportal and centrilobular regions.

Table II. Activities of the marker enzymes alanine aminotransferase (ALT) and glutamine synthetase (GS) as well as levels of apo P-450IIE1 and P-450IIE1 mRNA in rat hepatocytes isolated from periportal (pp) or centrilobular (cl) regions. The enzyme activities and P-450 values are given in % of the level in periportal preparations. Results are means ± SD of three experiments.

	pp	cl	ratio cl/pp
P-450IIE1 mRNA	100±30	390±210	3.9
P-450IIE1	100±18	280±40	2.8
ALT *	100	47	0.47
GS *	100	3850	39

*/ The specific activities of pp ALT and GS were 0.33±0.06 μmol/mg, min and 0.50±0.25 nmol/mg, min, respectively.

about a two times more preference in centrilobular eluates (not shown). After ethanol-treatment, P-450 IIE1 was selectively induced in eluates from the centrilobular region (Table I). Accordingly, the amount of this apoprotein was now more than 60-fold higher in the centrilobular eluates. On the other hand, phenobarbital-treatment of the rats caused the induction of IIB1/2 in both centrilobular and periportal regions, as indicated by an about a 2-fold higher concentration of this protein in centrilobular eluates (Table I).

Supernatants from isolated centrilobular cells contained about 3-fold higher amounts of P-450IIE1 as compared to the corresponding periportal preparations (Table II). The difference in the apoprotein level was in good agreement with the amount of P-450IIE1 mRNA detected by Northern blot from the two cell types (Fig. 1 & Table II).

The intralobular distribution of P-450IIE1 in livers from control rats was confirmed by immunohistochemistry. As seen from Fig. 2, this type of P-450 was almost exclusively distributed in centrilobular regions. About 30-40 % of the liver cells expressed immunodetectable amounts of P-450IIE1.

DISCUSSION

Taken together, examination of rat livers by immunohistochemistry, immunoanalysis of isolated periportal and centrilobular hepatocytes as well as Western blot analysis of digitonin lysates from the two different lobular regions, demonstrate that the ethanol-inducible form of P-450 (IIE1) is expressed almost exclusively in zone 3 surrounding the terminal hepatic venulas. The differences between isolated cells were less pronounced and is probably a consequence of less complete separation, in comparsion to the other two methods, of the two cell types during isolation. This is obvious from the observed smaller difference in the distribution of the marker enzymes (Table II vs. Table I). The finding of a similar relative distribution of apo P-450IIE1 and P-450IIE1 mRNA between isolated periportal and centrilobular cells indicates that the P-450IIE1 gene is preferentially expressed in centrilobular hepatocytes, although mechanisms involving stabilization of P-450IIE1 mRNA cannot be excluded. The factors responsible for this

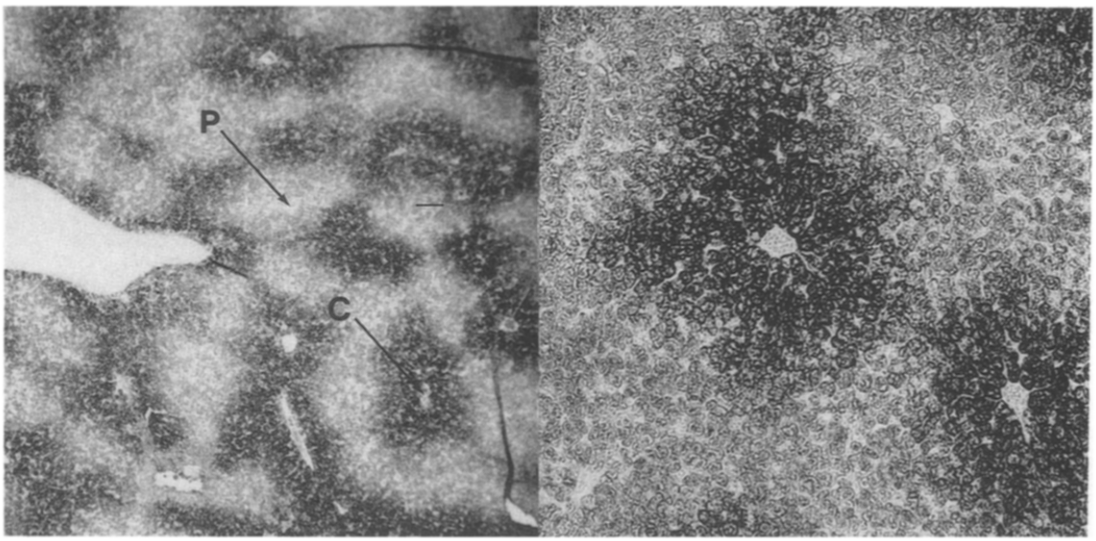

Fig. 2. Immunocytochemical analysis of intralobular distribution of cytochrome P-450IIE1 using polyclonal antibodies. Sections were from untreated rats. Positive staining is apparent in the centrilobular areas (C) in contrast to periportal areas (P) which display no or little staining.

selective expression are unknown and are the subjects for future investigations. One might speculate that the sinusoidal gradients of the concentrations of oxygen, nutrients and hormones could contribute to this phenomenon. To our knowledge, GS (19) and possibly carbonic anhydrase II (20) are the only other enzyme proteins demonstrated to exhibit a similar exclusively centrilobular expression.

P-450IIE1 was selectively induced in the centrilobular region by ethanol treatment. This was in contrast to the phenobarbital-dependent induction of P-450IIB1/2, which also occurred in the periportal region (Table I). It also contrasts to results of previous investigations regarding the inductive capability of phenobarbital, methylcholantrene, trans-stilbene oxide and cyanopregnenolone on several other different forms of cytochromes P-450, all of which are induced in both lobular regions (5, 21). Since much evidence has been given for a post-translational induction mechanism of P-450IIE1 by ethanol in liver (16, 18, 22, 23), one might suggest that the regioselective induction of IIE1 by ethanol is caused by stabilization of proteins already expressed in centrilobular hepatocytes. Thus, the same regioselective effect is to be expected by all other compounds, such as imidazole, 2-propanol, dimethylsulfoxide etc., that are believed to induce P-450IIE1 at a post-translational level (16, 18).

The heterogeneous distribution of P-450IIE1 might have potentially important implications in terms of mechanisms responsible for regioselective toxicity of various xenobiotics. Thus, efficient substrates for P-450IIE1, such as ethanol, carbon tetrachloride, acetaminophen and chloroform are all known to cause selective destruction of the centrilobular region of the liver (2, 24-26). Furthermore, P-450IIE1 has a unique property in that it very effectively reduces oxygen also in the absence of substrates (27), with the resulting formation of oxygen species capable of initiating lipid peroxidation (28). These findings suggest that induction of P-450IIE1 following treatment with e.g. ethanol or isoniazid or as a consequence of severe fasting or diabetes (cf (29)), might be of toxicological importance due to its regiospecificity in the liver.

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