TETRAHYDROFURANE - AN INHIBITOR FOR ETHANOL-INDUCED LIVER MICROSOMAL CYTOCHROME P450

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Summary

Liver microsomes from ethanol-pretreated rats have been compared with microsomes from male and female controls and phenobarbital- and benzpyrene-pretreated rats. The O-dealkylation activity for 7-ethoxycoumarin was enhanced after all treatments. Metyrapone selectively inhibited the activity after pretreatment with phenobarbital and naphthoflavone blocked the activity after benzpyrene treatment. Ethanol and even more so tetrahydrofurane inhibited specifically the O-dealkylation in microsomes from ethanol-pretreated rats. Only in these microsomes tetrahydrofurane produced a pronounced ligand-type optical difference spectrum and concomitantly a new low-spin cytochrome P450 species in the EPR-spectrum. According to inhibition experiments, liver microsomes from male and female rats have a different pattern of cytochrome P450 species.

Lipophilic organic compounds are known to be metabolized by the cytochrome P450 dependent monoxygenase system present in liver microsomes (1). It is now established that after pretreatment of animals with polycyclic hydrocarbons a cytochrome P450 (P448) is induced which differs from that in controls (2,3,4). On the basis of differences in spectra and monoxygenase activities, we have postulated a general heterogeneity of microsomal cytochrome P450 (5) which is now supported by a variety of other findings (6,7,8). It is still a matter of discussion whether a microsomal cytochrome species exists that can oxidize ethanol and also is induced by ethanol as postulated by Rubin et al. (9) and by Comai and Gaylor (10). We therefore, have investigated whether ethanol-pretreated rats can also perform the O-

dealkylation of the fluorogenic substrate 7-ethoxycoumarin (11) and if so, whether this activity was different from that found in controls or after phenobarbital and benzpyrene induction.

Methods

Male and female Sprague-Dawley rats (150-180 g) were used. Sodium phenobarbital was given i.p. in a daily dose of 80 mg/kg body weight for three days. 3,4-Benzpyrene was dissolved in corn oil and applied i.p. in a dose of 20 mg/kg for two days. Ethanol was given in the drinking water (30 % v/v) together with sucrose (30 % w/v) for a period of three weeks together with a standard diet (Altromin®). Microsomes were prepared as described previously (12) and protein was determined according to Gornall et al. (13). The test for 7-ethoxycoumarin O-dealkylation has been described (11) and spectra were recorded in an Aminco DW-2 spectrophotometer.

Results and Discussion

Livers of ethanol-pretreated rats had decreased about 50 % in weight compared to controls but contained a higher specific activity for the O-dealkylation of 7-ethoxycoumarin. The K_m-value for the monoxygenase reaction was found to be 3.2×10^{-5}M and therefore was significantly different from the corresponding K_m-value from benzpyrene (Bp)-induced rats (1.5×10^{-6}M) but similar to the K_m obtained after phenobarbital (Pb) pretreatment (2.7×10^{-5}M) (8). However, with the use of the inhibitor metyrapone large differences became apparent (Table).

As already reported, metyrapone inhibited the activity in microsomes from Pb-treated rats, and naphthoflavone (7,8-benzoflavone) proved to be rather specific for blocking the O-dealkylation in Bp-pretreated rats (8). It was interesting that the male controls but not the females could also be inhibited by metyrapone.

The inhibition values listed in the Table could not be presented as K_i-values since straight lines were not obtained in reciprocal plots as expected as a consequence of different cytochrome P450 species.

Table. Effect of inhibitors on the liver microsomal O-dealkylation of 7-ethoxycoumarin after various pretreatments of rats. The values represent means (± SD) of 6-8 rats.

Pretreatment		None (Controls)		Phenobarbital	Benzpyrene	Ethanol	
Sex		♂	♀	(♂ + ♀)[1]	(♂ + ♀)[1]	♂	♀
Specific Ativity nMol/mg Prot. min.		0,2 ± 0,1	0,05 ± 0,01	2,2 ± 0,3	5,4 ± 1	0,4 ± 0,1	0,15 ± 0.02
% Inhibition by Metyrapone	2×10^{-6}	30 ± 2	0 ± 1	29 ± 2	0 ± 1	15 ± 3	0 ± 1
	4×10^{-6}	45 ± 6	1 ± 1	39 ± 3	0 ± 1	23 ± 3	0 ± 1
	8×10^{-6}	50 ± 7	2 ± 1	60 ± 5	0 ± 1	28 ± 4	0 ± 1
	2×10^{-5}	52 ± 5	5 ± 2	72 ± 6,5	0 ± 1	34 ± 4	1 ± 1
% Inhibition by 7,8-Benzoflavone	$2,5 \times 10^{-6}$	-3 ± 1	0 ± 0,5	0 ± 0,5	12 ± 3	0 ± 1	0 ± 1
	5×10^{-6}	-3 ± 1	0 ± 1	2 ± 1	42 ± 5	0 ± 1	0 ± 1
	1×10^{-5}	3 ± 2	5 ± 2	3 ± 1	79 ± 4	0 ± 1	0 ± 1
	2×10^{-5}	5 ± 2,5	12 ± 3	3 ± 1	90 ± 3	0 ± 1	0 ± 1
% Inhibition by Tetrahydrofurane	1×10^{-3}	4 ± 1	14 ± 3	0 ± 1	0 ± 1	8 ± 2	27 ± 2
	2×10^{-3}	6 ± 1	24 ± 4	0 ± 1	1 ± 0,5	12 ± 3	36 ± 4
	5×10^{-3}	10 ± 2	29 ± 7	3 ± 1	2 ± 1	24 ± 3	65 ± 6
	1×10^{-2}	15 ± 3	48 ± 10	4 ± 1	2 ± 1	32 ± 4	79 ± 5

[1] No significant differences observed

When looking for an O-dealkylation inhibitor in ethanol-treated rats, ethanol itself turned out to be effective, but also a series of small molecules with oxygen functions were tried. Among those compounds tetrahydrofurane (THF) proved to have the highest affinity and specificity. THF at a concentration of 10^{-2}M blocks the activity by 80 %, whereas, no effect was seen in microsomes from Pb- or Bp-treated rats. Microsomes from female controls were significantly more sensitive to this inhibitor than those from males just opposite to the results with metyrapone.

Fig. 1 represents the optical difference spectrum obtained with THF in microsomes from male controls and ethanol-pretreated female rats. The higher magnitude of the peak at 413 nm after pretreatment indicates a possible relation to the altered inhibition characteristics of the preparation. Ethanol gives a similar spectrum in agreement with other findings (9). Upon titration with THF, isosbestic points at 342 and 398 nm are obtained and a K_s-value for this spectral change of 3.6×10^{-4}M can

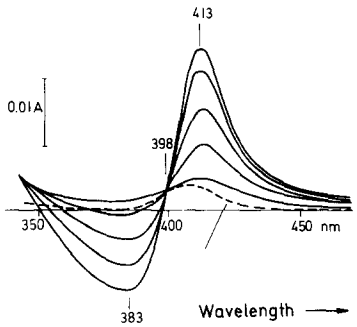

Fig. 1. Difference spectra of liver microsomes from ethanol-pretreated female rats with increasing concentrations of THF. The solid curves correspond to 0.01, 0.12, 0.36, 0.84 and 8.4 mM THF. The dashed line represents the spectrum obtained with 10^{-2}M THF in microsomes from male control rats. Both microsomal suspensions contained 2.5 nMol cytochrome P450/ml.

be calculated. The EPR-spectrum is also modified in the presence of THF showing the appearance of a new low-spin signal with g_x and g_z values at 2.50 and 1.90 respectively (Fig. 2). The spectral data tentatively could be explained by an interaction of the free electron pair of the oxygen atom in THF at the sixth coordination position of a special cytochrome P450 species.

The presented data strongly favor the existence of such a species after ethanol treatment of rats. It remains to be established whether this form is related to the THF-inhibited O-dealkylation activity in female control rats. Nevertheless, it is obvious that the well-known sex differences of drug metabolism in rats (14,15) can be mainly explained by differences in the pattern of the microsomal cytochrome P450 species. A similar conclusion was derived very recently from a study on various drug oxidations under chronic ethanol administration

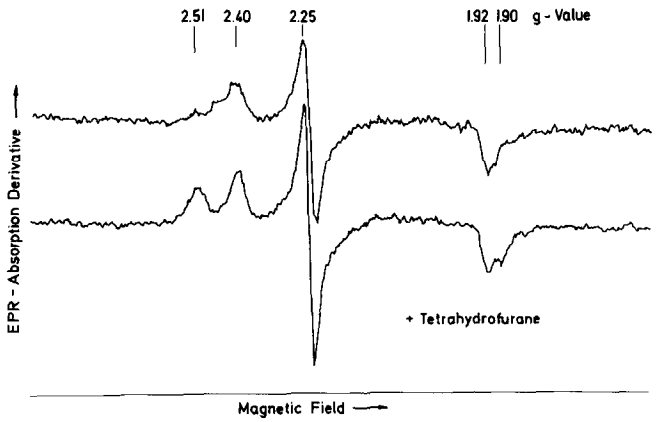

Fig. 2. Effect of THF on the EPR-spectrum of liver microsomes from ethanol-pretreated female rats at -180°C. Upper curve: microsomes containing 25 μM cytochrome P450. Lower curve: 2 x 10^{-2}M THF added. EPR parameters: modulation amplitude 10 G, modulation frequency 100 KHz, microwave power 60 mW, microwave frequency 9.16 GHz.

to male and female rats (16). The properties of the various cytochrome P450 species with special reference to ethanol metabolism are currently under investigation.

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