Stereochemical Studies on the Cytochrome P-450 and Hydroxyl Radical Dependent Pathways of 2-Butanol Oxidation by Microsomes from Chow-Fed, Phenobarbital-Treated, and Ethanol-Treated Rats†

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ABSTRACT: Microsomes have the potential to oxidize alcohols by two pathways, one dependent on hydroxyl radicals (·OH) and the other dependent on cytochrome P-450 in which ·OH does not seem to be involved. The possibility that these two pathways may display differences in stereospecificity was evaluated by comparing the oxidation of (+)-2-butanol, (−)-2-butanol, and racemic 2-butanol. Microsomes oxidized 2-butanol to 2-butanone by a reaction which was partially sensitive to carbon monoxide and to competitive ·OH scavengers. Desferrioxamine, which completely blocks the production of ·OH by microsomes, inhibited the oxidation of ethanol by about 60%, while the oxidation of 2-butanol and 1-butanol was decreased by only 30%. V_{max} values for the oxidation of ethanol, 1-butanol, and 2-butanol were 17.7, 6.2, and 23.8 nmol min^{-1} (mg of protein)$^{-1}$, respectively, in the absence of desferrioxamine and 5.9, 4.7, and 13.6 nmol min^{-1} (mg of protein)$^{-1}$, respectively, in the presence of desferrioxamine. 2-Butanol appears to be a particularly good alcohol substrate for the cytochrome P-450 dependent pathway of alcohol oxidation. Chronic ethanol consumption, which induces the microsomal alcohol oxidizing system, resulted in a 3-fold increase in the rate of 2-butanol oxidation. Most of this increment reflected an increased rate of metabolism by the cytochrome P-450 pathway. A type 2 binding spectrum was observed for the interaction of 2-butanol with microsomes from ethanol-fed rats, but not with controls. Hence, 2-butanol appears to be a very good alcohol substrate for the ethanol-inducible cytochrome P-450. The rates of oxidation of racemic 2-butanol, (+)-2-butanol, and (−)-2-butanol were the same for (a) model ·OH-generating systems, (b) microsomes from chow-fed rats, and (c) microsomes from phenobarbital-treated rats. These results suggest that the ·OH-dependent and the cytochrome P-450 dependent pathways of alcohol oxidation by these microsomes do not display stereospecificity. By contrast, microsomes from the chronic ethanol-fed rats catalyzed the oxidation of the (+) enantiomer at rates twice that of the (−) enantiomer of 2-butanol. These differences were observed only for the cytochrome P-450 dependent pathway. Thus, the stereochemical preference displayed by microsomes from chronic alcohol fed rats may be due to the induction of a particular cytochrome P-450. Stereochemical studies may be of value in elucidating the mechanism and the identification of the ethanol-inducible cytochrome P-450.

Isolated rat liver microsomes can oxidize a variety of alcohols to their corresponding aldehydes (Teschke et al., 1975). Recent experiments have suggested that microsomes have the potential to oxidize alcohols by two pathways (Cederbaum & Dicker, 1983; Winston & Cederbaum, 1983a,b). A role for a cytochrome P-450 dependent pathway in the oxidation of alcohols by microsomes has been demonstrated in reconstituted systems containing NADPH, NADPH-cytochrome P-450 reductase, cytochrome P-450, and phospholipids (Teschke et al., 1977; Joly et al., 1977; Miwa et al., 1978). This cytochrome P-450 dependent pathway of ethanol oxidation appears to be independent of a significant role for oxygen radicals (Cederbaum & Dicker, 1983; Winston & Cederbaum, 1983b). In the presence of iron, e.g., iron–ethylenediaminetetraacetic acid (iron–EDTA), microsomes have the potential to oxidize alcohols by a second pathway, which involves interaction of the alcohols with hydroxyl radicals (·OH) generated during microsomal electron transfer. During microsomal NADPH-dependent electron transport, several ·OH scavenging agents were oxidized to products identical with those found with chemical ·OH-generating systems (Cohen & Cederbaum, 1980). Oxidation of ·OH scavengers was inhibited by ethanol, and in turn, the oxidation of ethanol was lowered by ·OH scavengers (Cohen & Cederbaum, 1980; Cederbaum et al., 1978). Thus, in the presence of iron–EDTA, ·OH plays some role in the microsomal pathway of alcohol oxidation (Feierman & Cederbaum, 1983).

The current report represents an attempt to determine the stereospecific nature of the ·OH pathway and the cytochrome P-450 pathway of alcohol oxidation by induced and uninduced microsomes. The stereospecificity of the alcohol-oxidizing system by uninduced microsomes was previously studied with stereospecifically labeled ethanol (Gang et al., 1973; Corrall et al., 1974). Microsomes contain contaminating catalase, and ethanol is a good substrate for the peroxidatic activity of catalase. Because no inhibitors of catalase were used in those studies, it is possible that the stereospecificity observed for (1*R*)-ethanol may, in fact, have been due to the stereospecificity that catalase displays. In addition, it is unlikely that a potent oxidant such as a free ·OH would display stereospecificity. It is now generally believed that in biological systems, a highly reactive oxygen species such as ·OH would not be free in solution but rather may reflect a "·OH-like" species that is bound. Therefore, stereospecificity studies could provide some insight into the nature of the ·OH or ·OH-like species generated by the microsomes.

To evaluate the stereospecificity of the microsomal alcohol oxidation system, experiments with racemic 2-butanol, (+)-2-butanol, and (−)-2-butanol were undertaken. 2-Butanol

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inhibited the metabolism of aminopyrine (Dietz et al., 1981) by isolated microsomes, suggesting that the alcohol interacts with cytochrome P-450. As will be described under Results, 2-butanol, relative to ethanol and 1-butanol, appeared to be a particularly good substrate for the cytochrome P-450 pathway, and it was anticipated that if any stereospecificity existed, it would likely occur via the cytochrome P-450 pathway. Studies were, therefore, initiated to determine whether 2-butanol is metabolized by microsomes, to identify the role of ·OH and cytochrome P-450 in 2-butanol oxidation, and to compare the oxidation of (+)- and (−)-2-butanol by microsomes from chow-fed control rats, as well as rats treated with the inducers phenobarbital or ethanol.

Materials and Methods

Hepatic microsomes were prepared from male Sprague-Dawley rats as described previously (Cederbaum & Dicker, 1983). In some cases, rats were pretreated with sodium phenobarbital [80 mg (kg body weight)$^{-1}$ day^{-1}] for 3 days. Chronic ethanol treatment was carried out by feeding rats for 4 weeks a nutritionally adequate liquid diet in which ethanol provided 36% of the total calories. Pair-fed littermates consumed the same diet except that carbohydrate isocalorically replaced ethanol (Lieber & DeCarli, 1970b). The microsomes were washed once and suspended in 125 mM KCl. Protein was determined by the method of Lowry et al. (1951).

The oxidation of alcohols was assayed at 37 °C in 25-mL Erlenmeyer flasks containing 100 mM potassium phosphate, pH 7.4, 10 mM sodium pyrophosphate, 10 mM $MgCl_2$, 10 mM glucose 6-phosphate, 2.3 units of glucose-6-phosphate dehydrogenase, 0.4 mM $NADP^+$, 0.1 mM EDTA, 1.0 mM azide, and about 2.5 mg of microsomal protein in a final volume of 1.0 mL. Except in the kinetic studies, the final concentration of 1-butanol and 2-butanol was 33 mM, while the final concentration of ethanol was 50 mM. The reaction was initiated with glucose 6-phosphate plus glucose-6-phosphate dehydrogenase and terminated by the addition of hydrochloric acid (final concentration of 300 mM). In cumene hydroperoxide dependent systems, the reaction was initiated with the hydroperoxide (final concentration of 1 mM), and glucose 6-phosphate plus glucose-6-phosphate dehydrogenase was omitted from the reaction mixture. The oxidation products of ethanol (acetaldehyde), 1-butanol (butyraldehyde), and 2-butanol (2-butanone) were measured by gas chromatography. The flasks were sealed with serum stoppers and incubated at 60 °C for 20 min. A 1.0-mL aliquot of the headspace was directly injected into a Hewlett-Packard Model 5750 gas chromatograph equipped with a 6-ft 5% Carbowax 20 M Haloport F 30-60 column. The injection temperature and oven temperature were 100 and 50 °C, respectively. The carrier gas used was nitrogen at a flow rate of 35 mL/min. Under the above conditions, the retention times (minutes) were as follows: ethanol = 0.95, acetaldehyde = 0.40, 1-butanol = 3.74, butyraldehyde = 0.80, 2-butanol = 1.80, and 2-butanone = 0.95.

Peak areas were quantitated by comparison to the appropriate standard curve. All values reported have been corrected for "zero time" controls in which HCl was added before microsomes or where microsomes were omitted from the flasks.

The binding spectrum of 2-butanol was determined by the method of Peterson et al. (1971) using a Perkin-Elmer Model 554 dual-beam spectrophotometer. The concentration of 2-butanol was 128 mM, and microsomal protein was approximately 0.4 mg.

All chemicals were of the highest grade available. The buffers (except $MgCl_2$) were passed through a Chelex-100 column (Bio-Rad Laboratories, Richmond, CA) to remove contaminating iron. Desferrioxamine (Desferal) was obtained from CIBA Pharmaceutical Co. (Summit, NJ); (+)-2-butanol and (−)-2-butanol were obtained from Fairfield Chemical Co., Inc. (Blythewood, SC), or from Pfaltz & Bauer, Inc. (Stamford, CT). Racemic 2-butanol was also obtained from Pfaltz & Bauer.

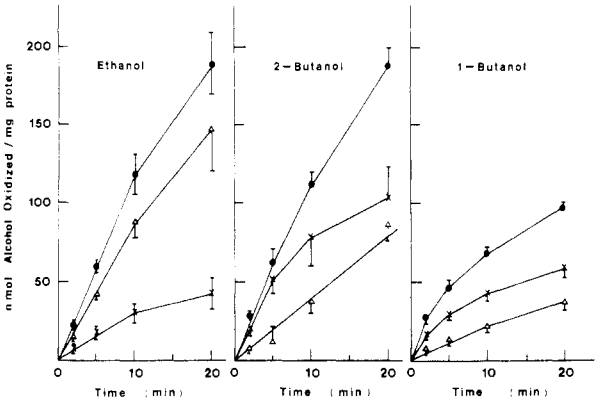

FIGURE 1: Time course for the oxidation of ethanol, 2-butanol, and 1-butanol by microsomes from chow-fed rats. The oxidation of the alcohols was determined as described under Materials and Methods in the absence and presence of 0.25 mM desferrioxamine. The ·OH-dependent rate was calculated by subtracting the rate in the presence of desferrioxamine from the total rate. Results are from three experiments. (●) Rate in the absence of desferrioxamine (total rate). (×) Rate in the presence of desferrioxamine. (△) ·OH-dependent rate.

All values refer to the mean ± standard error of the mean (SEM). Statistical analyses were performed by Student's *t* test. The number of experiments is indicated in the tables or in the figure legend.

Results

Experiments described here, unless otherwise specified, refer to the racemic 2-butanol and microsomes isolated from chow-fed rats.

Oxidation of 2-Butanol by Rat Liver Microsomes. Initial experiments showed that 2-butanol was oxidized to 2-butanone by rat liver microsomes in an NADPH-dependent manner. Azide, an inhibitor of catalase, increased the oxidation of 2-butanol by about 2-fold. This should be contrasted to the decreased oxidation of ethanol (which is a substrate for the peroxidatic activity of catalase) produced by azide (Cederbaum et al., 1978). 2-Butanol was also not oxidized in the presence of purified catalase plus an H_2O_2-generating system. These results suggest that 2-butanol is not an effective substrate for the peroxidatic activity of catalase.

Figure 1 compares the time-dependent oxidation of 2-butanol with that of ethanol and 1-butanol in the absence and presence of desferrioxamine. The rate in the absence of desferrioxamine reflects the oxidation of alcohols by two pathways, the ·OH-dependent and the cytochrome P-450 dependent system. Desferrioxamine, a potent iron-chelating agent, nearly completely abolished the production of ·OH by microsomes (Cederbaum & Dicker, 1983). The desferrioxamine-resistant rate of oxidation of alcohols appears to reflect the cytochrome P-450, ·OH-independent pathway of alcohol oxidation. The difference between the rates in the absence and presence of desferrioxamine is taken as the ·OH-dependent rate of oxidation of alcohols. All three alcohols are oxidized in a time-dependent manner, in both the absence and presence of desferrioxamine. Total rates of oxidation for ethanol and 2-butanol were linear for 10 min, while the rates in the

Table I: Kinetic Constants for the Oxidation of Ethanol, 2-Butanol, and 1-Butanol in the Absence and Presence of Desferrioxamine[a]

	−desferrioxamine		+desferrioxamine	
substrate	K_m (mM)	V_{max} (nmol min^{-1} mg^{-1})	K_m (mM)	V_{max} (nmol min^{-1} mg^{-1})
ethanol	14.9	17.7	10.4	5.9
2-butanol	19.1	23.8	18.6	13.6
1-butanol	4.4	6.2	5.8	4.7

[a] K_m and V_{max} values were obtained from a least-squares linear regression of Lineweaver–Burk plots of data which determined the rates of oxidation of alcohols as a function of the concentration of the alcohol. Alcohol concentrations ranged from 5 to 100 mM in the case of ethanol and 2-butanol and from 2 to 50 mM in the case of 1-butanol. Results are from six experiments with ethanol or 2-butanol as substrate and three experiments with 1-butanol as substrate.

Table II: Effect of Hydroxyl Radical Scavenging Agents and Desferrioxamine on Microsomal Oxidation of 2-Butanol[a]

addition	concn (mM)	2-butanol oxidation [nmol min^{-1} (mg of protein)$^{-1}$]	effect (%)	p
control		13.7 ± 0.8		
benzoate	30	10.5 ± 0.5	−23	<0.02
benzoate	60	9.9 ± 0.4	−28	<0.05
Me_2SO	30	9.7 ± 0.8	−29	<0.01
Me_2SO	60	7.1 ± 0.2	−48	<0.001
desferrioxamine	0.25	8.9 ± 0.8	−36	<0.05

[a] The oxidation of racemic 2-butanol by microsomes from chow-fed rats was assayed as described under Materials and Methods. The results represent the averages of three or four experiments.

presence of desferrioxamine were linear for 10 min with ethanol and 5 min with 2-butanol, but less with 1-butanol. The total rates of oxidation of ethanol and 2-butanol were similar at all time points, whereas the oxidation rate of 1-butanol was considerably less at the later time points (Figure 1). Desferrioxamine produced a significantly greater inhibition of ethanol oxidation than of the oxidation of 2- or 1-butanol. With ethanol as the substrate, it appeared that the ·OH pathway was responsible for about 60–70% of the total oxidation, whereas with 2- and 1-butanol, the ·OH pathway comprised about 30% of the total oxidation. Of significance is the observation that, in the presence of desferrioxamine, 2-butanol was oxidized at approximately twice the rate of the other alcohols. This suggested that 2-butanol could serve as a relatively better substrate for cytochrome P-450 than the other alcohols.

The relative kinetic constants for alcohol oxidation were determined by using Lineweaver–Burk plots. With ethanol and 2-butanol, the alcohol substrate concentrations used were 5, 10, 25, 50 and 100 mM, while with 1-butanol, substrate concentrations of 2, 5, 10, 25, and 50 mM were utilized. Table I shows that, in the absence of desferrioxamine, the K_m for ethanol and 2-butanol was the same, while the K_m for 1-butanol was much lower. The K_m values for the three alcohols were not significantly changed in the presence of desferrioxamine. The V_{max} for 2-butanol was greater than that for ethanol and 1-butanol. In particular, the V_{max} for 2-butanol oxidation in the presence of desferrioxamine was especially higher than the V_{max} for the other two alcohols (Table I). Thus, the kinetic data also suggest that 2-butanol is a particularly good substrate for the cytochrome P-450 pathway.

To further characterize the oxidation of 2-butanol by rat liver microsomes, studies involving the effect of ·OH scavengers as well as modifiers of cytochrome P-450 function were carried out. Table II shows that benzoate and dimethyl sulfoxide (Me_2SO) produced some inhibition of 2-butanol oxidation.

Table III: Effect of Aniline and CO on the Oxidation of 2-Butanol by Rat Liver Microsomes[a]

	rate of oxidation of 2-butanol [nmol min^{-1} (mg of protein)$^{-1}$]			
	−desferrioxamine		+desferrioxamine	
addition	rate	effect (%)	rate	effect (%)
control	13.8 ± 1.3		8.5 ± 1.1	
2 mM aniline	9.9 ± 1.3	−28	5.1 ● 0.5	−40
control (100% air)	14.2 ± 2.1		10.8 ± 1.7	
30% N_2/70% air	13.2 ± 1.9	−7	9.3 ± 1.5	−15
30% CO/70% air	10.3 ± 1.7	−27	5.6 ± 0.9	−48

[a] The oxidation of racemic 2-butanol was assayed in the absence and presence of 0.25 mM desferrioxamine. Results are from either three (aniline) or six to seven (N_2 and CO) experiments.

The extent of inhibition by the competitive ·OH scavengers was about the same as that found with desferrioxamine (Table II). Previous experiments showed that ethanol oxidation was reduced 50–70% by the addition of desferrioxamine and competitive ·OH scavengers such as benzoate and Me_2SO (Cederbaum & Dicker, 1983; Cederbaum et al., 1978), which should be contrasted to the lower inhibition seen with 2-butanol (Table II). Taken as a whole, these results suggest some role for ·OH in the oxidation of 2-butanol, although relative to ethanol, 2-butanol appears to be oxidized preferably by the cytochrome P-450 pathway. The somewhat greater extent of inhibition by 60 mM Me_2SO may be due, in part, to the recently demonstrated interaction of high concentrations of Me_2SO with cytochrome P-450 (Morgan et al., 1981, 1982).

Aniline has been shown to be a good substrate for the cytochrome P-450 isozyme(s) which are induced by ethanol (Rubin et al., 1970; Morgan et al., 1982). Table III shows that aniline inhibited the oxidation of 2-butanol. In a similar manner, a mixture of 30% CO/70% air produced inhibition of 2-butanol oxidation. The inhibitory effects of aniline and CO were even greater with respect to the cytochrome P-450 pathway alone (rates in the presence of desferrioxamine; Table III). A 30% CO/70% air mixture produced 47% inhibition of the oxidation of a typical drug substrate, aminopyrine, which is similar to that seen with 2-butanol.

Organic hydroperoxides such as cumene hydroperoxide react directly with cytochrome P-450 to form an oxygenated complex that catalyzes the metabolism of drugs (Rahimtula & O'Brien, 1974, 1975). Cumene hydroperoxide is also capable of oxidizing ethanol to acetaldehyde in a microsomal system and by purified cytochrome P-450 (Rahimtula & O'Brien, 1977). The oxidation of typical ·OH scavengers, however, was not supported by cumene hydroperoxide (Cederbaum, 1983). Cumene hydroperoxide did support microsomal oxidation of 2-butanol to 2-butanone. Desferrioxamine had no effect on this organic hydroperoxide-dependent oxidation of 2-butanol, suggesting that ·OH is not involved in this system. Rates were 7.8 ± 0.3 and 8.3 ± 0.1 nmol min^{-1} (mg of protein)$^{-1}$ in the absence and presence of desferrioxamine, respectively.

Oxidation of 2-Butanol by Microsomes from Ethanol-Fed Rats. Chronic ethanol consumption has been shown to increase the microsomal oxidation of ethanol (Lieber & DeCarli, 1970a; Teschke et al., 1977). A cytochrome P-450 isozyme distinct from those induced by phenobarbital or 3-methylcholanthrene was induced by ethanol (Ohnishi & Lieber, 1977; Koop et al., 1982), which proved to have high activity for the metabolism of ethanol and aniline (Ohnishi & Lieber, 1977; Morgan et al., 1982). Microsomes from ethanol-fed rats catalyzed the oxidation of ·OH scavengers at rates that were higher than control rates (Klein et al., 1983). Since 2-butanol

Table IV: Oxidation of Racemic 2-Butanol and (+)- and (−)-2-Butanol by Liver Microsomes from Chow-Fed, Phenobarbital-Treated, and Ethanol-Fed Rats and Pair-Fed Controls[a]

microsomes	substrate	rate of oxidation [nmol min^{-1} (mg of protein)$^{-1}$]		
		total	+desferrioxamine	·OH component
chow-fed (n = 4–6)	racemic 2-butanol	12.6 ± 1.9	8.5 ± 1.1	4.1 ± 0.8
	(+)-2-butanol	10.2 ± 1.0	6.6 ± 0.6	3.6 ± 0.7
	(−)-2-butanol	11.1 ± 1.8	7.6 ± 1.2	3.5 ± 0.7
phenobarbital (n = 3)	racemic 2-butanol	11.3 ± 1.3	7.8 ± 1.0	3.5 ± 0.9
	(+)-2-butanol	10.8 ± 1.6	6.8 ± 0.9	4.0 ± 1.1
	(−)-2-butanol	9.6 ± 0.8	7.2 ± 1.3	2.4 ± 0.6
chronic ethanol (n = 3)	racemic 2-butanol	37.2 ± 1.8	29.5 ± 3.2	7.7 ± 1.7
	(+)-2-butanol	34.0 ± 2.8	27.9 ± 2.5	6.1 ± 0.6
	(−)-2-butanol	20.7 ± 2.7[b]	14.0 ± 1.3[b]	6.7 ± 1.2
pair fed (n = 3)	racemic 2-butanol	15.2 ± 2.0	9.4 ± 0.3	5.8 ± 1.8
	(+)-2-butanol	14.0 ± 1.7	8.3 ± 0.6	5.7 ± 1.6
	(−)-2-butanol	11.6 ± 2.1	6.5 ± 0.2[b]	5.1 ± 2.0

[a] The oxidation of racemic 2-butanol and (+)- and (−)-2-butanol by microsomes from chow-fed controls, from phenobarbital-treated rats, from ethanol-fed rats, and from their pair-fed controls was assayed in the absence (total) and presence of 0.25 mM desferrioxamine. [b] $p < 0.01$ with respect to the (+) isomer or the racemic 2-butanol.

appeared to be an effective substrate for the cytochrome P-450 pathway of alcohol oxidation, we compared its oxidation by microsomes from chronic ethanol-fed rats and their pair-fed controls. Chronic ethanol consumption resulted in an increase in the rate of oxidation of 2-butanol; rates of 2-butanone production were 31 ± 6 and 13 ± 2 nmol min^{-1} (mg of protein)$^{-1}$ for microsomes from ethanol-fed rats and pair-fed controls, respectively (n = 6 pairs, $p < 0.01$). The ·OH-dependent oxidation of 2-butanol was slightly increased, but most of the increase in the oxidation of 2-butanol reflected an increase in the cytochrome P-450 pathway since rates in the presence of 0.25 mM desferrioxamine were 23 ± 4 and 8 ± 1 nmol min^{-1} (mg of protein)$^{-1}$ for microsomes from ethanol-fed rats and pair-fed controls, respectively (n = 6 pairs). Thus, 2-butanol appears to be an effective substrate for the chronic ethanol-inducible cytochrome P-450 isozyme(s).

Substrates for cytochrome P-450 typically display type 1 or type 2 binding spectra. 2-butanol displayed a type 2 binding spectrum with microsomes from chronic alcohol-fed rats, with a maximum at about 415 nm and a minimum at about 380 nm (data not shown). By contrast, a clear type 1 or type 2 spectrum with 2-butanol was not observed with microsomes from the pair-fed controls or from chow-fed controls (data not shown). Although the significance of binding spectra is difficult to interpret, these results may suggest some special interaction of 2-butanol with microsomes from the chronic ethanol-fed rats.

Stereospecificity Studies with (+)- and (−)-2-Butanol. After the oxidation of racemic 2-butanol by rat liver microsomes had been characterized, the oxidation of (+)-2-butanol, (−)-2-butanol, and racemic 2-butanol in the absence and presence of desferrioxamine was compared. Optical rotation analysis verified that both isomers had equal but opposite rotations.

Prior to initiating studies of the stereospecific nature of 2-butanol oxidation in the more complex microsomal system, experiments were carried out in two well-characterized model ·OH-generating systems, the iron-catalyzed oxidation of ascorbate (Cohen, 1977) and the Fenton reaction (Walling, 1975). Both systems oxidized the (+)-2-butanol and (−)-2-butanol at similar rates (data not shown).

Results shown in Table IV indicate that microsomes isolated from chow-fed control rats oxidized (+)-2-butanol, (−)-2-butanol, and racemic 2-butanol at similar rates, both in the absence and in the presence of desferrioxamine Subtraction of the rates in the presence of desferrioxamine from the total rates to yield the ·OH-dependent rates indicated that the ·OH-dependent rates were also the same for (+)-2-butanol, (−)-2-butanol and racemic 2-butanol.

In view of the multiplicity of cytochrome P-450 in normal microsomes, it was considered that perhaps an induced system might display different results. Rats were treated with phenobarbital, a well-known inducer of certain cytochrome P-450 isozymes. The rate of oxidation of 2-butanol by microsomes from phenobarbital-treated rats was similar to rates found with microsomes from controls (Table IV). No stereospecificity was observed either in the absence or in the presence of desferrioxamine for microsomes isolated from phenobarbital-treated rats (Table IV).

Experiments were next carried out with microsomes isolated from the chronic ethanol-fed rats. As shown in Table IV, whereas the oxidation of the (+)-2-butanol stereoisomer was the same as that of racemic 2-butanol, the (−)-2-butanol stereoisomer was oxidized at a significantly lower rate. This difference was especially notable in the presence of desferrioxamine, where the (+) isomer was oxidized at a 2-fold greater rate than the (−) isomer (Table IV). Thus, in these microsomes, the cytochrome P-450 dependent pathway of alcohol oxidation displays some stereospecificity. The ·OH-dependent pathway of alcohol oxidation was, however, not stereospecific (Table IV).

It was also interesting to note that there was a trend toward some discrimination against the (−) isomer by microsomes from the pair-fed animals although the differences were not as striking as those seen after chronic ethanol treatment (Table IV). No such discrimination was noted for the ·OH-dependent pathway.

Discussion

The current study characterizes in detail the oxidation of 2-butanol by rat liver microsomes. The inhibition of 2-butanol oxidation by desferrioxamine and by competitive ·OH scavengers suggests some role for ·OH in the oxidation of 2-butanol. However, the contribution of the ·OH-dependent pathway toward the overall oxidation of 2-butanol appears to be considerably less than when ethanol is the substrate for the microsomal alcohol oxidizing system. The kinetic studies shown in Figure 1 and Table I indicate that 2-butanol is a particularly effective alcohol substrate for the cytochrome P-450 pathway of oxidation of alcohols. Longer chain alcohols may be more effective substrates for cytochrome P-450 as a consequence of their greater hydrophobicity. Indeed, the K_i for inhibition of drug metabolism by alcohols correlated with their partition coefficient (Cohen & Mannering, 1973; Khanna

et al., 1980). 1-Butanol also appears to be oxidized primarily by the cytochrome P-450 pathway; however, the V_{max} for 1-butanol oxidation is much smaller than that for 2-butanol oxidation. This lower rate of 1-butanol oxidation occurs despite the fact that the K_m for 1-butanol is lower than that of 2-butanol (Table I). It appears that the position of the oxidizable hydroxyl group may be important in determining the overall turnover of the alcohol substrate. In this context, *tert*-butyl alcohol is oxidized at much lower rates than either 1- or 2-butanol, and *tert*-butyl alcohol appears either to be a very poor substrate or not to be a substrate at all for cytochrome P-450 (Cederbaum et al., 1983).

Chronic consumption of ethanol increases the rate of oxidation of ethanol by microsomes (Lieber & DeCarli, 1970a; Teschke et al., 1977). The oxidation of 2-butanol is also increased after ethanol consumption. This increase is largely insensitive to desferrioxamine, which suggests that the increase is primarily due to enhanced metabolism of 2-butanol by the cytochrome P-450 pathway and that 2-butanol, as compared to ethanol, may be a more effective substrate for the alcohol-inducible cytochrome P-450. This may prove to be of value in studies concerning the mechanism of alcohol oxidation and the stoichiometry between utilization of NADPH and O_2 and product formation.

Of value in the choice to study 2-butanol was the fact that this alcohol exists as two stereochemical isomers, the (+) and (−) enantiomers, and 2-butanol is not a substrate for catalase. Hence, a comparison of the oxidation of (+)-2-butanol and (−)-2-butanol may provide information concerning the mechanism of microsomal oxidation of alcohols and on the nature of the ·OH generated during microsomal electron transfer. It would be anticipated that a ·OH "free in solution" would not demonstrate stereochemical discrimination, whereas a "bound ·OH" might preferentially oxidize one of the stereoisomers. On the other hand, an enzymatic reaction such as that catalyzed by cytochrome P-450 might display stereospecificity. Cytochrome P-450 has been shown to display stereospecificity toward certain drugs (Jenner & Testa, 1973; Yang & Gelboin, 1976; Deutsch et al., 1979; Kaminsky et al., 1980; Porter et al., 1981; Guengerich et al., 1982; Thakker et al., 1977, 1982) and has been implied in the stereospecificity of others (Ames & Frank, 1982). In the case of alcohols, it was noted that the microsomal alcohol-oxidizing system showed the same stereospecificity that catalase exhibits with regard to ethanol (Gang et al., 1973; Corrall et al., 1974), and since inhibitors of catalase such as azide were not used in these experiments, the question as to whether or not the microsomal alcohol-oxidizing system (independent of catalase) is indeed stereospecific still remained to be determined.

Rates of oxidation of (+)-2-butanol (−)-2-butanol, and racemic 2-butanol were not significantly different from each other when microsomes from chow-fed rats or phenobarbital-treated rats were utilized. No stereospecificity was observed in the absence or presence of desferrioxamine with either microsomal preparations. This suggests that neither the total oxidation rate, the cytochrome P-450 dependent rate, nor the ·OH-dependent rate is stereospecific for 2-butanol with these microsomes. The lack of stereospecificity by the ·OH-dependent mechanism suggests that the active oxygen species either is similar to that found in the model chemical systems (e.g., free in solution) or perhaps is bound in such a manner that it can still react equally well with either isomer of 2-butanol.

The rate of oxidation of 2-butanol by microsomes from the phenobarbital-treated rats is the same on a per milligram of microsomal protein basis as that found with uninduced control microsomes. This suggests that the phenobarbital-induced cytochromes P-450 may not be an especially effective catalyst for the oxidation of alcohols. By contrast, chronic ethanol consumption results in a 3-fold increase in the rate of 2-butanol oxidation by the microsomes. In this case, it was noted that (+)-2-butanol was preferentially oxidized in comparison with its enantiomer. The difference in the rate of oxidation is approximately a 2-fold increase for the (+) isomer, both in the absence and in the presence of desferrioxamine. The difference between the desferrioxamine-inhibited rate and the total rate (which yields the ·OH rate) shows no stereospecificity. Hence, the stereochemical effects seen in microsomes from alcohol-treated rats appear to be due to the induction of a distinct cytochrome P-450 isozyme after chronic consumption of ethanol. The fact that complete stereospecificity is not observed in these experiments may be due to the existence of various populations of cytochrome P-450 in isolated microsomal preparations, some of which may display stereospecificity with regard to the oxidation of 2-butanol and some which may not. It would, therefore, be of interest to determine the stereospecific nature of the microsomal alcohol-oxidizing pathway with purified cytochrome P-450 preparations, especially the alcohol-inducible cytochrome P-450. To date, however, it has not been possible to purify this enzyme to homogeneity from rat liver.

It was interesting to note that microsomes from pair-fed rats displayed some preference for (+)-2-butanol oxidation over the (−) isomer by the cytochrome P-450 dependent pathway. While the discrimination for the (+) isomer was not as sharp as that seen with microsomes from ethanol-fed rats, there was nonetheless a difference which is not observed with microsomes from chow-fed rats. This suggests the possibility that some differences in the cytochrome P-450 isozyme composition may exist between microsomes isolated from rats fed the pair-fed control diet and chow-fed controls.

The sharp discrimination for (+)-2-butanol by microsomes from alcohol-induced animals compared to the other systems tested may prove to be of value in the identification of a distinct cytochrome P-450 induced by ethanol.

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Registry No. Ethanol, 64-17-5; 1-butanol, 71-36-3; (±)-2-butanol, 15892-23-6; (+)-2-butanol, 4221-99-2; (−)-2-butanol, 14898-79-4; cytochrome P-450, 9035-51-2; hydroxy radical, 3352-57-6.

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