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INCREASED SENSITIVITY OF THE MICROSOMAL OXIDATION OF ETHANOL TO INHIBITION BY PYRAZOLE AND 4-METHYLPYRAZOLE AFTER CHRONIC ETHANOL TREATMENT

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Abstract—Pyrazole and 4-methylpyrazole, inhibitors of the oxidation of ethanol by alcohol dehydrogenase, also inhibit microsomal metabolism of ethanol. The inhibitory effectiveness of these agents was increased in microsomes isolated from rats treated chronically with ethanol as compared to microsomes from pair-fed controls or from rats treated with other cytochrome P-450 inducers such as phenobarbital or 3-methylcholanthrene. Pyrazole and 4-methylpyrazole produced type II binding spectra with all the microsomal preparations. However, there was an increased affinity (lower K_s value) for these agents by the microsomes from the ethanol-fed rats. A correlation between K_s values and inhibitory effectiveness against ethanol oxidation by the various microsomal preparations could be observed. This suggests that an increase in affinity, which may reflect the induction of an alcohol-preferring isozyme of cytochrome P-450, is responsible for the increased inhibitory effectiveness of pyrazole and 4-methylpyrazole towards ethanol oxidation by microsomes after chronic ethanol treatment. One difference between pyrazole and 4-methylpyrazole was the increased affinity and inhibitory effectiveness of the latter but not the former with microsomes from rats treated with 3-methylcholanthrene. This could be due to the ability of 4-methylpyrazole, compared to pyrazole, to interact with and induce several isozymes of cytochrome P-450. Pyrazole and 4-methylpyrazole are often utilized to evaluate ethanol metabolism by alcohol-dehydrogenase-dependent and -independent pathways. However, the sensitivity of microsomal ethanol oxidation to inhibition by these agents, especially after chronic ethanol treatment, would suggest that their use in this regard is complex and could tend to underestimate the contribution of the microsomal pathway towards the metabolic tolerance found after ethanol treatment.

Pyrazole and 4-methylpyrazole are potent inhibitors of alcohol dehydrogenase and of ethanol metabolism [1–3]. In addition, these agents also display a variety of *in vivo* and *in vitro* interactions with liver microsomes. *In vivo* treatment of rats with pyrazole or 4-methylpyrazole appears to result in the induction of an alcohol-preferring cytochrome P-450 isozyme as shown by the increased microsomal oxidation of alcohols [4, 5], and drugs such as aniline and dimethylnitrosamine [6–9], as well as interaction with antibody raised against the purified cytochrome P-450-3a isozyme [10]. *In vitro*, pyrazole and 4-methylpyrazole react with microsomes to give rise to type II binding spectra [11, 12], and they inhibit the oxidation of a variety of drugs including ethanol [13, 14].

Recent experiments characterized the *in vitro* inhibition of the microsomal oxidation of ethanol by pyrazole and 4-methylpyrazole. It was observed that the inhibitory effectiveness of these agents is increased with microsomes isolated from rats previously treated with either pyrazole or 4-methylpyrazole [15]. This increased sensitivity was suggested to reflect an increased interaction with the cytochrome P-450 isozyme(s) induced by the pyrazole or 4-methylpyrazole treatment. Since this cytochrome P-450 isozyme(s) has properties similar to the isozymes induced by chronic ethanol treatment [5, 9, 10], the current experiments were carried out in order to evaluate the inhibitory effectiveness of pyrazole and 4-methylpyrazole against ethanol oxidation by microsomes isolated from rats treated with ethanol and their pair-fed controls, and to compare this inhibitory effectiveness with microsomes isolated from other classic inducers of certain cytochrome P-450 isozymes such as phenobarbital and 3-methylcholanthrene (3-MC). Since pyrazole and 4-methylpyrazole are often utilized to assess the contribution of alcohol-dehydrogenase-dependent and -independent (largely microsomal) pathways towards the overall metabolism of ethanol, an increased sensitivity of the microsomal oxidation of the ethanol pathway to inhibition by these agents after chronic ethanol treatment would complicate interpretation of this assessment.

MATERIALS AND METHODS

Male, Sprague–Dawley rats weighing about 135 g were fed a Lieber–DeCarli liquid diet for 3–4 weeks in which ethanol provided 36% of total caloric intake, protein 18%, fat 35% and carbohydrate 11% [16]. Pair-fed controls consumed the same diet except that carbohydrate isocalorically replaced ethanol.

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During the last 2 weeks, the rats consumed about 18 g ethanol per kg body wt per day. Prior to the day of sacrifice, the rats received their respective diets *ad lib*. Other groups of rats were either injected once a day i.p. with a suspension of 3-MC in corn oil (20 mg/ml) for 3 days at a dose of 25 mg/kg body wt, or with an equivalent volume of corn oil. An additional group of rats consumed a 0.1% (w/v) sodium phenobarbital solution for 10 days. The rats were starved overnight prior to being killed. Liver microsomes were prepared by differential centrifugation, suspended in 125 mM KCl, and washed once prior to use.

The oxidation of ethanol by microsomes was assayed at 37° using a reaction mixture containing 100 mM potassium phosphate, pH 7.4, 10 mM $MgCl_2$, 0.4 mM $NADP^+$, 1 mM sodium azide (to inhibit catalase), 55 mM ethanol and about 2 mg microsomal protein in a final volume of 1 ml. The reaction was initiated by the addition of a mixture of glucose-6-phosphate (final concentration of 10 mM) plus 1 unit of glucose-6-phosphate dehydrogenase and terminated after 5 min by the addition of 0.3 ml of 1 N HCl. The production of acetaldehyde was determined by a head-space gas chromatography procedure [5]. All values were corrected for zero-time controls in which the HCl was added before the NADPH-generating system.

Since ethanol can be oxidized by the hydroxyl radical to produce acetaldehyde [17], and pyrazole and 4-methylpyrazole are potent hydroxyl radical scavenging agents [14], the possible involvement of oxy-radicals in ethanol oxidation by microsomes was minimized by avoiding the use of EDTA and by passing the buffers and water used to prepare solutions through columns of Chelex-100 resin to remove metals such as iron. Under these reaction conditions, we have estimated that most of the ethanol (about 90%) is oxidized by a cytochrome P-450-dependent pathway that is independent of a role for hydroxyl radicals [18].

Binding spectrum experiments with pyrazole and 4-methylpyrazole as substrates were carried out using a Perkin–Elmer model 554 dual beam spectrophotometer. Approximately 2 mg of microsomal protein was suspended in 6 ml of 0.1 M phosphate buffer, pH 7.4. The samples were divided in half and, after a base line correction, various concentrations of pyrazole or 4-methylpyrazole were added to the sample cuvette and the spectrum was scanned over the wavelength range of 450 to 350 nm.

All values refer to mean ± SEM. Statistical analyses between pairs (Table 2) were carried out by Student's *t*-test (two-tailed). For evaluation of the response to various doses of either pyrazole or 4-methylpyrazole (Tables 1 and 3), statistical analyses were conducted using repeated measurement analysis of variance (ANOVA). The number of experiments is indicated in the table or figure legends. Pyrazole was obtained from Pfaltz & Bauer (Waterbury, CT) and 4-methylpyrazole was from the Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of the highest grade available.

RESULTS

Microsomal oxidation of ethanol after chronic ethanol treatment. The rate of ethanol oxidation by microsomes was increased more than 4-fold after chronic ethanol treatment when results were expressed per mg protein (Table 1). The content of cytochrome P-450 was increased 2-fold (pair-fed controls, 0.68 ± 0.10 nmol/mg protein; chronic ethanol, 1.37 ± 0.01 nmol/mg protein, N = 3). Thus, part of the increase in ethanol oxidation was due to the increase in content of cytochrome P-450, whereas the additional increase was probably due to the

Table 1. Effects of pyrazole and 4-methylpyrazole on the microsomal oxidation of ethanol after chronic ethanol treatment

Addition	Concn (mM)	Rate of ethanol oxidation (nmol/min/mg microsomal protein)		Effect of addition (%)	
		Chronic ethanol	Pair-fed control	Chronic ethanol	Control
None		13.0 ± 1.9	2.7 ± 0.4		
Pyrazole	0.25	8.9 ± 0.8†	ND*	−32	ND
	0.50	7.3 ± 1.2†	2.8 ± 0.2	−44	+4
	1.0	6.8 ± 0.7†	2.7 ± 0.3	−48	0
	3.0	ND	1.9 ± 0.6	ND	−30
4-Methylpyrazole	0.25	5.6 ± 0.8‡	2.3 ± 0.1	−57	−15
	0.50	5.2 ± 0.7‡	2.2 ± 0.3	−60	−19
	1.0	4.1 ± 0.4‡	2.1 ± 0.3†	−68	−22
	3.0	ND	2.0 ± 0.4†	ND	−24

The oxidation of 55 mM ethanol by microsomes isolated from rats treated chronically with ethanol or their pair-fed controls was determined in the presence of the indicated concentrations of either pyrazole or 4-methylpyrazole. Results (mean ± SEM) are from three pairs of animals.

* ND = not determined.

† $P < 0.05$.

‡ $P < 0.01$.

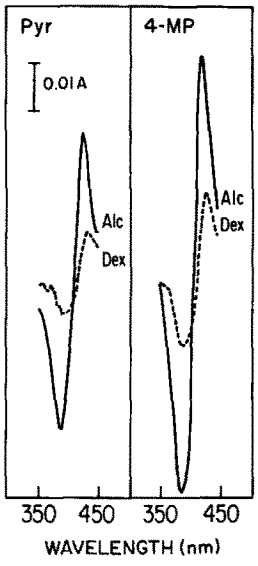

Fig. 1. Binding spectrum of the interaction of pyrazole (Pyr) or 4-methylpyrazole (4-MP) with microsomes from rats treated chronically with ethanol (Alc) or pair-fed controls (dextrose-fed). Results from a typical pair of animals are depicted. The final concentration of pyrazole or 4-methylpyrazole was 2.67 mM.

induction of an alcohol-preferring cytochrome P-450 isozyme.

Various concentrations of pyrazole and 4-methylpyrazole were added to the microsomes, and the effect on ethanol oxidation was determined. Microsomes isolated from rats chronically fed ethanol appeared to be particularly sensitive to inhibition by both pyrazole and 4-methylpyrazole as the addition of low concentrations of these agents produced significant inhibition of ethanol oxidation (Table 1). By contrast, microsomes from the pair-fed controls were considerably more resistant to inhibition by either pyrazole or 4-methylpyrazole (Table 1). It is interesting to note that, in both preparations of microsomes, 4-methylpyrazole was a somewhat more effective *in vitro* inhibitor of the microsomal oxidation of ethanol than was pyrazole. Thus, analogous to previous results with microsomes isolated from rats treated with pyrazole or 4-methylpyrazole [15], treatment with ethanol appears to result in a microsomal preparation in which the oxidation of ethanol is especially sensitive to inhibition by the *in vitro* addition of pyrazole or 4-methylpyrazole.

Binding of pyrazole and 4-methylpyrazole to microsomes after ethanol treatment. Pyrazole and 4-methylpyrazole bind to liver microsomes to produce a type II spectral change with a peak at about 429 nm and a trough at about 392 nm [11, 12]. A typical spectrum for the interaction of these agents with microsomes from pair-fed controls and after chronic ethanol treatment is shown in Fig. 1. The magnitude of the binding spectral change associated with the interaction of either pyrazole or 4-methylpyrazole with microsomes was increased considerably after chronic ethanol treatment (Fig. 1). Similar increases in the binding spectrum as compared to saline controls were noted previously with microsomes isolated from rats treated with pyrazole or 4-methylpyrazole [15].

Various concentrations of pyrazole and 4-methylpyrazole were added to the microsomes from the ethanol-fed rats and their pair-fed controls, and the magnitude of the binding spectrum, as reflected by the change in absorbance (429–392 nm) was determined. The magnitude of the spectral change increased as the concentration of pyrazole or 4-methylpyrazole was increased (results not shown). A Hanes–Wolfe plot of these data was linear with both pyrazole (Fig. 2, panel A) and 4-methylpyrazole (Fig. 3, panel A) as substrates with both microsomal preparations. Spectral dissociation constants (K_s) and maximal spectral changes (ΔA_{max}) from the $A_{429} - A_{392}$ were calculated from linear regression analyses of these plots and are summarized in Table 2. Chronic ethanol consumption resulted in an increase in affinity by microsomes for methylpyrazole

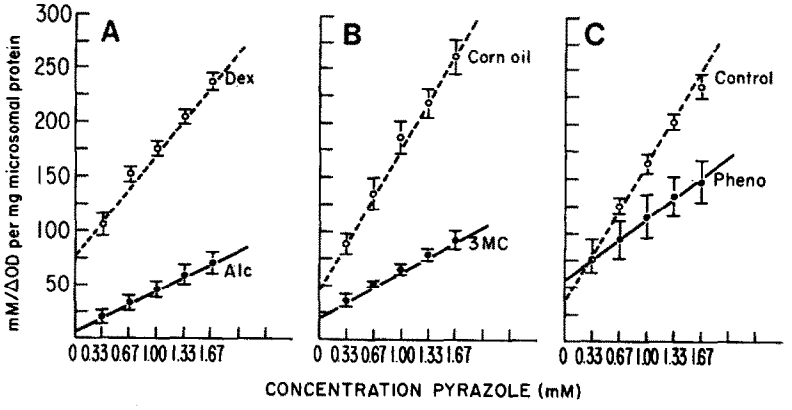

Fig. 2. Hanes–Wolfe plots ($s/\Delta A$ versus s) for the interaction of pyrazole with microsomes isolated from rats treated with ethanol (Alc), pair-fed controls (dextrose-fed), 3-methylcholanthrene (3-MC), corn-oil controls, phenobarbital (Pheno) or chow controls. Various concentrations of pyrazole (0.33 to 1.67 mM) were added to the microsomes, and the change in absorbance (429–392 nm) was recorded. Results are from three to four experiments for each treatment group.

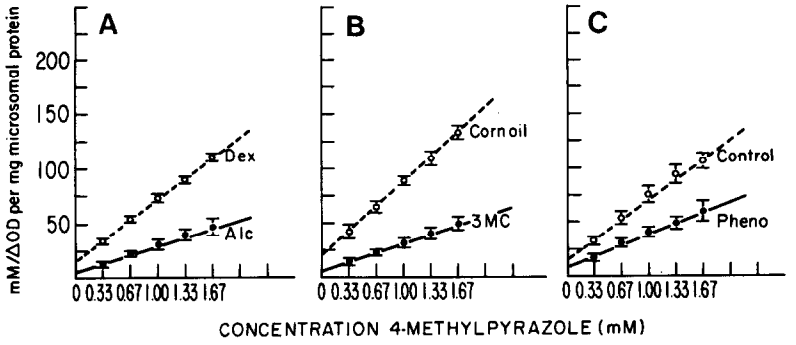

Fig. 3. Hanes–Wolfe plots ($s/\Delta A$ versus s) for the interaction of 4-methylpyrazole with microsomes. The abbreviations are as described in the legend to Fig. 2. Results are from three to four experiments for each treatment group.

and, especially, pyrazole (Table 2). There was also an increase in the maximal spectral change associated with the binding of these agents; however, this increase was due to the elevated content of cytochrome P-450 after ethanol consumption since no changes were observed when ΔA_{max} values were expressed per nmol cytochrome P-450 (Table 2).

Microsomal oxidation of ethanol after treatment with phenobarbital or 3-methylcholanthrene. For comparative purposes, the effects of treatment with other inducers of cytochrome P-450 isozymes on the inhibition of ethanol oxidation by pyrazole and 4-methylpyrazole were studied. The cytochrome P-450 isozymes induced by phenobarbital or 3-methylcholanthrene differ from the isozymes induced by ethanol [19, 20] or pyrazole [9, 10]. The oxidation of ethanol was higher with microsomes isolated from rats treated with 3-MC compared to corn oil controls (Table 3). However, this increase was due to an increase in the total content of cytochrome P-450. Contents of cytochrome P-450 (nmol/mg protein) were about 0.6, 1.6 and 1.5 for microsomes isolated from corn-oil-, 3-MC- and phenobarbital-treated rats respectively. Microsomes from the phenobarbital-treated rats were especially ineffective in catalyzing the oxidation of ethanol, especially if rates were expressed on a per nmol cytochrome P-450 basis.

When pyrazole was utilized as the *in vitro* inhibitor of ethanol oxidation, corn-oil- and 3-MC-treated rats displayed a similar sensitivity to inhibition, whereas the microsomes from the phenobarbital-treated rats were more resistant to inhibition (Table 3). When 4-methylpyrazole was utilized as the *in vitro* inhibitor of ethanol oxidation, microsomes from corn-oil- and phenobarbital-treated rats displayed a similar sensitivity to inhibition, whereas the microsomes from

Table 2. Parameters associated with the binding of pyrazole and 4-methylpyrazole to microsomes

Substrate	Treatment	K_s (mM)	ΔA_{max}/mg protein	ΔA_{max}/nmol cytochrome P-450
Pyrazole	Pair-fed (3)	0.90 ± 0.12	0.0108 ± 0.0003	0.0165 ± 0.0022
	Ethanol (3)	0.15 ± 0.03	0.0259 ± 0.0047	0.0189 ± 0.0034
		$P < 0.005$	$P < 0.03$	NS
	Control (3)	0.32 ± 0.02	0.0083 ± 0.0004	0.0123 ± 0.0020
	Phenobarbital (3)	1.14 ± 0.29	0.0185 ± 0.0004	0.0122 ± 0.0010
		$P < 0.005$	$P < 0.001$	NS
	Corn oil (4)	0.39 ± 0.08	0.0077 ± 0.0004	0.0136 ± 0.0007
	3-MC (4)	0.51 ± 0.08	0.0240 ± 0.0026	0.0148 ± 0.0014
		NS	$P < 0.001$	NS
4-Methylpyrazole	Pair-fed (3)	0.27 ± 0.03	0.0174 ± 0.0005	0.0265 ± 0.0039
	Ethanol (3)	0.11 ± 0.02	0.0382 ± 0.0053	0.0279 ± 0.0035
		$P < 0.03$	$P < 0.02$	NS
	Control (3)	0.32 ± 0.02	0.0165 ± 0.0011	0.0219 ± 0.0020
	Phenobarbital (3)	0.32 ± 0.01	0.0323 ± 0.0040	0.0211 ± 0.0060
		NS	$P < 0.001$	NS
	Control (4)	0.34 ± 0.08	0.0151 ± 0.0003	0.0266 ± 0.0006
	3MC (4)	0.17 ± 0.02	0.0383 ± 0.0052	0.0236 ± 0.0030
		$P\ 0.10 > P > 0.05$	$P < 0.005$	NS

The various binding constants, expressed as mean ± SEM, were derived from linear regressions of the Hanes–Wolfe plots shown in Figs. 2 and 3. The number of pairs of animals is shown in parentheses. Statistical analyses were made between the treatment group (ethanol, phenobarbital or 3-methylcholanthrene) and the appropriate control group (pair-fed, chow-fed, corn-oil-treated). NS = not significant.

Table 3. Effects of pyrazole and 4-methylpyrazole on the microsomal oxidation of ethanol after treatment with phenobarbital or 3-methylcholanthrene

Addition	Concn (mM)	Rate of ethanol oxidation (nmol/min/mg microsomal protein)			Effect of addition (%)		
		Corn-oil	3-MC	Phenobarbital	Corn-oil	3-MC	Phenobarbital
None		3.9 ± 0.4	6.5 ± 1.0	2.9 ± 0.4			
Pyrazole	0.5	ND*	5.2 ± 1.1	2.5 ± 0.5	ND	−20	−14
	1.0	2.8 ± 0.5†	4.6 ± 1.0†	2.4 ± 0.6	−27	−29	−17
	3.0	2.3 ± 0.6†	3.9 ± 0.8‡	2.2 ± 0.7	−40	−40	−22
	10.0	2.1 ± 0.4†	2.8 ± 0.6‡	1.5 ± 0.7‡	−46	−56	−46
4-Methyl-pyrazole	0.5	ND	3.7 ± 1.0†	2.2 ± 0.1§	ND	−43	−24
	1.0	2.5 ± 0.5†	3.2 ± 0.8‡	1.8 ± 0.2‡	−35	−51	−38
	3.0	2.4 ± 0.5†	3.0 ± 0.6‡	1.7 ± 0.2‡	−39	−55	−40
	10.0	1.8 ± 0.4‡	2.1 ± 0.4‡	1.3 ± 0.1‡	−52	−67	−55

The oxidation of 55 mM ethanol by microsomes isolated from rats treated with 3-methylcholanthrene or phenobarbital or corn-oil was determined in the presence of the indicated concentrations of either pyrazole or 4-methylpyrazole. Results (mean ± SEM) are from three to four experiments.

* ND = not determined.

† $P < 0.05$.

‡ $P < 0.01$.

§ $P\ 0.10 > P > 0.05$.

the 3-MC-treated rats appeared to be somewhat more sensitive to inhibition (Table 3). With all three types of microsomal preparations, significant inhibition of ethanol oxidation required higher concentrations of either pyrazole or 4-methylpyrazole than that found to be necessary to inhibit ethanol oxidation by microsomes from ethanol-treated rats (Table 1) or rats treated with pyrazole or 4-methylpyrazole [15].

Pyrazole and 4-methylpyrazole reacted with microsomes isolated from rats treated with phenobarbital or 3-MC (and their respective controls) to produce type II spectral changes similar to those depicted in Fig. 1. The magnitude of the binding spectral change increased as the concentration of either pyrazole or 4-methylpyrazole was increased, and Hanes–Wolfe plots with all microsomal preparations evaluated were linear with both pyrazole (Fig. 2, panels B and C) and 4-methylpyrazole (Fig. 3, panels B and C). The various spectral binding constants are summarized in Table 2. Treatment with 3-MC did not alter the affinity by microsomes for pyrazole relative to corn-oil controls, whereas treatment with phenobarbital decreased this affinity (Table 2). Treatment with 3-MC, but not phenobarbital, increased the affinity for 4-methylpyrazole (Table 2). Maximal spectral changes associated with the binding of either pyrazole or 4-methylpyrazole were enhanced after treatment with 3-MC or with phenobarbital when results were expressed per mg microsomal protein. However, similar to results found after chronic ethanol treatment, this increase was due to the increase in total content of cytochrome P-450 produced by the 3-MC or phenobarbital treatment. Treatment with 3-MC or phenobarbital did not produce any change in ΔA_{max} values when results were expressed per nmol total cytochrome P-450 (Table 2).

DISCUSSION

In vitro, pyrazole and 4-methylpyrazole can inhibit the microsomal oxidation of ethanol; however, the effectiveness of this inhibition varies with the nature of the microsomal preparation. Previous results demonstrated that pyrazole and 4-methylpyrazole are more effective inhibitors of ethanol oxidation by microsomes isolated from rats treated with these agents, as compared to saline controls [15]. Results in the present paper show that pyrazole and 4-methylpyrazole were also more effective inhibitors of ethanol oxidation by microsomes isolated from rats treated chronically with ethanol as compared with pair-fed controls. Treatment with pyrazole [4, 7–10, 21] and with 4-methylpyrazole [5, 9] induces an isozyme of cytochrome P-450 which appears to be similar in its properties to the isozyme induced by chronic ethanol treatment. The increased sensitivity of ethanol oxidation to inhibition by either pyrazole or 4-methylpyrazole after chronic ethanol treatment may reflect an increased interaction of pyrazole and 4-methylpyrazole with this inducible isozyme. Indeed, whereas pyrazole and 4-methylpyrazole produced type II binding spectra with microsomes from ethanol-fed rats and pair-fed controls, the magnitude of this spectral change was enhanced after ethanol treatment (Fig. 1). Analysis via Hanes–Wolfe plots revealed an increased binding affinity for pyrazole and 4-methylpyrazole by microsomes from ethanol-fed rats. This increase in affinity may explain the increased inhibitory effectiveness towards microsomal ethanol oxidation by pyrazole and 4-methylpyrazole after ethanol treatment.

Previous experiments with microsomes isolated from rats after pyrazole treatment for 2 days indicated that the K_s values for pyrazole and 4-methylpyrazole binding are 0.20 ± 0.02 and

0.12 ± 0.01 mM respectively (Table 3 of Ref. 15). These constants are identical to the K_s values for pyrazole and 4-methylpyrazole binding to microsomes after chronic ethanol treatment [pyrazole, $K_s = 0.15 \pm 0.03$; 4-methylpyrazole, $K_s = 0.11 \pm 0.02$ mM (Table 2)]. These identical affinities by microsomes after either pyrazole or chronic ethanol treatment are likely reflections of the induction of the same or of similar isozymes of cytochrome P-450 by these agents.

Substantially higher concentrations of pyrazole were required to inhibit ethanol oxidation by microsomes isolated from phenobarbital- or 3-MC-treated rats as compared to ethanol-treated rats (Tables 1 and 3). This could be due to a weaker interaction of pyrazole with the cytochrome P-450 isoenzymes induced by treatment with phenobarbital or 3-MC compared to the chronic ethanol-inducible isozyme. Results obtained via Hanes–Wolfe plots indicated that the binding affinity for pyrazole was highest with microsomes from ethanol-fed rats ($K_s = 0.15$ mM), intermediate with microsomes from the 3-MC-treated rats ($K_s = 0.51$ mM), and lowest with microsomes from the phenobarbital-treated rats ($K_s = 1.1$ mM). It is of interest to note that, while the affinity for pyrazole was similar in microsomes from 3-MC-treated, corn-oil and chow-fed controls, the microsomes from the phenobarbital-treated rats displayed a rather poor affinity for pyrazole. The extent of inhibition of microsomal ethanol oxidation by pyrazole appears to correlate with the K_s values for pyrazole, e.g. inhibition being greatest with microsomes from ethanol-treated rats, intermediate with microsomes from 3-MC- and corn-oil-treated rats, and poorest with microsomes from the phenobarbital-treated rats and the pair-fed controls (K_s for pyrazole = 0.90 mM, similar to that with the phenobarbital-treated). If the respective K_s values for pyrazole by the various microsomal preparations are plotted against the percent inhibition of ethanol oxidation produced by 1 mM pyrazole, a line can be drawn with a correlation coefficient of 0.86. Thus, the sensitivity of the microsomal ethanol oxidation system to pyrazole in the various preparations appears to reflect the affinity for, and interaction of, pyrazole with the cytochrome P-450 isozymes which predominate in that microsomal preparation.

Microsomes from phenobarbital-treated rats, corn-oil and pair-fed controls all displayed a similar sensitivity to inhibition by 4-methylpyrazole, whereas the microsomes from the 3-MC-treated (and ethanol-treated) rats were more sensitive. The extent of inhibition appears to correlate with the affinity of the microsomal preparation for 4-methylpyrazole. Values for K_s were identical for microsomes from phenobarbital-treated, corn-oil and pair-fed controls (0.30 mM), whereas a higher affinity for 4-methylpyrazole was found with the microsomes from the 3-MC-treated (0.17 mM) and ethanol-treated (0.11 mM) rats. A correlation coefficient of 0.84 was obtained from a plot of the respective K_s values for 4-methylpyrazole by the various microsomal preparations versus the percent inhibition of ethanol oxidation. Thus, analogous to results with pyrazole, the sensitivity of microsomal ethanol oxidation to 4-methylpyrazole reflects the nature and the interaction of the cytochrome P-450 isozymes in the microsomal preparation with 4-methylpyrazole. One interesting difference in response to pyrazole and 4-methylpyrazole is that 3-MC treatment resulted in an increased affinity for 4-methylpyrazole and a greater extent of inhibition of ethanol oxidation than that found with corn-oil controls, whereas affinity for pyrazole and inhibition by pyrazole was the same with corn oil and 3-MC treated. This enhanced affinity for 4-methylpyrazole but not pyrazole by 3-MC treatment could be a reflection of increasing hydrophobicity of the pyrazole analog. We have shown previously [9] that, whereas pyrazole administration *in vivo* does not alter total cytochrome P-450 content but results in an enrichment of a cytochrome P-450 isozyme with a molecular weight of about 52,000, *in vivo* administration of 4-methylpyrazole results in increased amounts of two or perhaps three cytochrome P-450 isozymes and increases the total content of cytochrome P-450. It therefore appears that 4-methylpyrazole may interact with and induce several isozymes of cytochrome P-450, whereas pyrazole may be less reactive in this regard.

Hepatic ethanol oxidation proceeds largely via an alcohol-dehydrogenase-dependent pathway. The microsomal ethanol oxidation pathway represents a minor pathway of ethanol metabolism, the significance of which has been the subject of much debate (reviewed in Refs. 22–24). Chronic consumption of ethanol increases the oxidation of ethanol by microsomes, and this increase has been suggested to contribute towards the metabolic tolerance found after chronic ethanol administration [25]. Pyrazole and 4-methylpyrazole are often utilized to assess the contribution of alcohol-dehydrogenase-dependent and -independent pathways towards the overall metabolism of ethanol. Implicit in the use of these agents is the underlying assumption that they only block the alcohol-dehydrogenase-dependent pathway. Since these agents also block microsomal oxidation of ethanol, use of pyrazole and 4-methylpyrazole to assess pathways of ethanol metabolism is complex, and could tend to underestimate the contribution by non-alcohol-dehydrogenase-dependent pathways. In view of the fact that ethanol oxidation by microsomes was considerably more sensitive to inhibition by pyrazole or 4-methylpyrazole after chronic ethanol treatment, use of these agents to evaluate the contributions made by different ethanol-oxidizing pathways after chronic ethanol consumption could be especially complicated, particularly with regard to underestimating the significance of the microsomal pathway. Other approaches, e.g. use of stereospecifically-labeled ethanol or isotope effects [26, 27], may be preferable to the use of pyrazole or 4-methylpyrazole in studies on pathways of ethanol oxidation.

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