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4-Methylpyrazole Inhibits Fatty Acyl Coenzyme Synthetase and Diminishes Catalase-Dependent Alcohol Metabolism: Has the Contribution of Alcohol Dehydrogenase to Alcohol Metabolism Been Previously Overestimated?

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SUMMARY

Alcohol dehydrogenase (ADH)-deficient deer mice were used as an animal model to investigate the effect of 4-methylpyrazole on alcohol metabolism. After intraperitoneal dosing of these mutant mice with 4-methylpyrazole, rates of ethanol and methanol metabolism *in vivo* were decreased significantly, by 41% and 35%, respectively. In perfused liver, rates of ethanol metabolism were also decreased up to 61% by 100 μM 4-methylpyrazole. Further, when livers were perfused with methanol, a selective substrate for catalase, rates of methanol metabolism were decreased by 64% by 4-methylpyrazole. It was further determined that 4-methylpyrazole administration caused negligible changes in total hepatic catalase activity and in rates of oxidation of ethanol by isolated microsomes; rather, it acts on catalase-dependent alcohol metabolism by limiting the supply of H_2O_2. In this study, 4-methylpyrazole inhibited fatty acyl CoA synthetase competitively in liver homogenates. Fatty acyl CoA synthetase is a key enzyme involved in the supply of substrate for peroxisomal oxidation of alcohols via catalase-H_2O_2. When palmitate was studied, rates of formaldehyde production from methanol were reduced competitively by 4-methylpyrazole; however, when the product palmitoyl CoA was used, the addition of 4-methylpyrazole did not alter activity. 4-Methylpyrazole also inhibited fatty acyl CoA synthetase activity measured directly from CoA disappearance. These data indicate that fatty acyl CoA synthetase is inhibited by 4-methylpyrazole, thus reducing the availability of H_2O_2 for catalase-dependent alcohol metabolism. Inhibition of methanol metabolism in deer mice expressing ADH indicates that this phenomenon also occurs in species with ADH. Taken together, these data support the hypothesis that the contribution of ADH to alcohol metabolism may have been previously overestimated.

Historically, the contribution of various metabolic pathways to alcohol metabolism has been estimated by utilizing inhibitors. Based on the sensitivity of ADH to alkyl pyrazoles, it is generally accepted that ADH is the predominant pathway of alcohol metabolism in both animals and humans. Theorell and Yonetani (1) first showed that alkyl pyrazoles were competitive inhibitors of ADH, and Lester *et al.* (2), Goldberg and Rydberg (3), and Bloomstrand and Theorell (4) determined that pyrazoles and their derivatives decreased ethanol metabolism *in vivo* 60–90%.

On the other hand, the specificity of alkyl pyrazoles for ADH has been questioned. Damgaard (5) reported that cytochrome P-450 was inhibited by 4-methylpyrazole, whereas Feytmans *et al.* (6) showed that pyrazole metabolites diminish the catalase-H_2O_2 complex 24 hr after treatment *in vivo* but had no effect on catalase *per se*. In spite of these reports, the idea that pyrazole and its derivatives are specific inhibitors of ADH has prevailed.

ADH^- deer mice represent a particularly useful model to evaluate the specificity of alkyl pyrazoles because they lack hepatic ADH yet metabolize alcohol at 50–70% of rates observed with deer mice that have ADH (7). Therefore, the purpose of this study was to evaluate the effect of 4-methylpyrazole on alcohol metabolism in ADH^- deer mice. The results indicate that 4-methylpyrazole inhibits the catalase pathway via actions on acyl CoA synthetase, raising the possibility that the contribution of ADH to alcohol metabolism has been overestimated previously.

Materials and Methods

Alcohol metabolism. Rates of ethanol or methanol metabolism were determined *in vivo* after administration of 2 g/kg ethanol or methanol (intraperitoneally) 30 min before 4-methylpyrazole (200 or 500 mg/kg, intraperitoneally) in saline. After treatment, deer mice were

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ABBREVIATIONS: ADH, alcohol dehydrogenase; ADH^-, alcohol dehydrogenase-deficient; ADH^+, alcohol dehydrogenase-expressing.

forced to breathe into a closed heated chamber (37°) for 30 sec, and 1 ml of breath was collected with a gas-tight syringe. Concentrations of ethanol or methanol were determined by gas chromatography, and rates of alcohol metabolism were calculated from linear decreases in blood alcohol concentration per unit time, as described previously (7).

Hemoglobin-free liver perfusion. Deer mouse livers were perfused with a miniature recirculating perfusion system routinely used in this laboratory (8). Livers were perfused with Krebs-Henseleit buffer containing 4% bovine serum albumin, to which 30 mM methanol or ethanol was added. Oxygen uptake was monitored continuously using a Clark-type electrode. 4-Methylpyrazole HCl (pH 7.6) was added at concentrations indicated in the table or figure legends. Samples of perfusate (0.05 ml) were collected every 10 min for standard determination of ethanol and methanol by head-space gas chromatography. Standards containing several concentrations of alcohol were analyzed along with the experimental samples. Rates of alcohol metabolism were calculated from the concentration differences per unit time, the volume of perfusate (17 ml), and the liver wet weight. All experiments were corrected for vaporization of alcohol from the liver.

Microsomal ethanol oxidation. Deer mice were sacrificed and hepatic microsomes were prepared by standard methods of differential centrifugation (9). Microsomal ethanol metabolism was estimated from the formation of acetaldehyde, as described in detail elsewhere (10). Rates of metabolism were based on protein concentrations determined according to the method of Lowry *et al.* (11).

Fatty acyl CoA synthetase activity. Livers from ADH$^-$ deer mice were homogenized using 4 volumes of 0.25 M sucrose buffer, and the resulting homogenate was added to a sucrose solution containing palmitate (0–2.0 mM) in the presence or absence of 4-methylpyrazole (0–5 mM), to yield a final concentration of 2–3 mg of protein/0.2 ml. After 45 min of incubation at room temperature, homogenates were incubated in 25-ml Erlenmeyer flasks at 37° for 10 min in 1.4 ml of Tris·HCl buffer, pH 8.3, containing 100 mM methanol, 0.1 mM CoA, 2.5 mM ATP, 5 mM $MgCl_2$, 200 μM NAD^+, 33 mM nicotinamide, 0.9 mg/ml bovine serum albumin, 0.01% Triton X-100, and 6.6 mM semicarbazide (12). In some experiments, palmitoyl CoA was substituted for palmitate, and ATP as well as CoA was omitted from the reaction mixture. Reactions were terminated by the addition of 0.1 ml of 40% trichloroacetic acid and the resulting mixture was centrifuged at 10,000 × *g* for 2 min. Supernatants were assayed for formaldehyde using a spectrophotometric procedure (13).

Direct assay of fatty acyl CoA synthetase. Livers from ADH$^-$ deer mice were removed, homogenized in 0.25 M sucrose, and centrifuged at 2000 × *g* for 10 min. The resulting supernatants were incubated for 45 min at room temperature after the addition of 4-methylpyrazole (0–10 mM). Rates of CoASH disappearance were determined using an assay described by Bar-Tana *et al.* (14). The assay mixture (0.25 ml) contained 0.15 M Tris·HCl, pH 7.4, 1 mg/ml Triton X-100, 2 mM EDTA, 50 mM $MgCl_2$, 20 mM ATP, 0.2 mM palmitate, 0.3 mM CoASH, and 3–12 μg of protein. Reactions were initiated by the addition of tissue. After incubation for 3 min at 37°, the reaction was terminated by the addition of 0.75 ml of 0.5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) in 0.1 M potassium phosphate buffer (pH 8.0) and the mixture was centrifuged. Blanks were prepared by adding the 5,5′-dithiobis-(2-nitrobenzoic acid) cocktail before the addition of protein. Activity was determined by the decrease in absorbance at 413 nm, using a molar extinction coefficient of $1.36 \times 10^4 \cdot cm^{-1}$.

Catalase activity. Catalase activity was determined using the method of Luck as described in Bergmeyer (15). Livers from ADH$^-$ deer mice were homogenized in 5 volumes of 0.07 mM phosphate buffer, pH 7.0, H_2O_2 was added, and the linear decline in absorbance per unit time was measured spectrophotometrically at 240 nm.

Results

A typical deer mouse liver perfusion study is depicted in Fig. 1. After a 35-min interval, ethanol (30 mM) was added and oxygen and ethanol uptake were measured. Basal rates of oxygen uptake were unchanged by ethanol, and rates of ethanol uptake were 125 μmol/g/hr in this example. Addition of 4-methylpyrazole at 70 min of perfusion caused a small transient decline in oxygen uptake over about 20 min but decreased ethanol uptake dramatically, to nearly 70 μmol/g/hr. On average, ethanol metabolism was decreased 60–65% by 100 μM 4-methylpyrazole (Table 1).

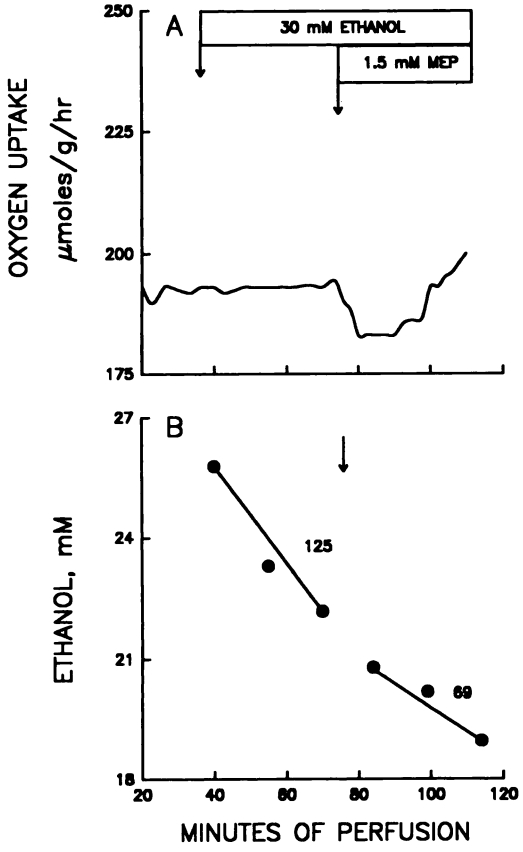

Fig. 1. Oxygen and ethanol uptake by the isolated perfused ADH$^-$ deer mouse liver. Livers were perfused in a recirculating perfusion system as described in Materials and Methods. Ethanol (30 mM) and 4-methylpyrazole (*MEP*) (1.5 mM) were added to perfusate containing 4% bovine serum albumin (pH 7.6) at 37°, as depicted by *horizontal bars with arrows* (A). Oxygen uptake was monitored using a Clark-type electrode, as described in Materials and Methods. Samples of perfusate were analyzed for ethanol as described in Materials and Methods. Rates of ethanol uptake by the perfused liver were 125 and 69 μmol/g/hr in the absence and presence of 4-methylpyrazole, respectively, in this example (B). Typical experiment.

The effect of 4-methylpyrazole on rates of oxidation of ethanol was also evaluated in microsomes isolated from ADH$^-$ deer mice. Control rates of microsomal oxidation of ethanol (i.e., 6.26 ± 0.25 nmol/mg/min) were not affected significantly by 10 mM 4-methylpyrazole (i.e., 5.97 ± 0.47 nmol/mg/min). Thus, 4-methylpyrazole had little effect on microsomal ethanol metabolism in tissue from ADH$^-$ deer mice.

Because methanol is a selective substrate for catalase in rodents (16), methanol metabolism was also measured in the isolated perfused deer mouse liver. An experimental design similar to that depicted in Fig. 1 was used to assess the effect of 4-methylpyrazole on the catalase system. Basal rates of oxygen uptake of 146 μmol/g/hr were unaffected by the addition of methanol (30 mM; data not shown). Rates of methanol uptake were also decreased significantly, by 60–65%, by 100 μM 4-methylpyrazole (Table 1).

TABLE 1

Effect of 4-methylpyrazole on ethanol and methanol uptake in the isolated perfused deer mouse liver

Livers from ADH⁻ deer mice were perfused at 37° (ethanol) or 30° (methanol), as described in Materials and Methods, using the experimental design depicted in Fig. 1. Average rates of alcohol uptake were calculated from 4–19 livers/group at 70 and 110 min of perfusion (Fig. 1). Alcohol concentrations were measured as described in Materials and Methods. Rates of alcohol metabolism were calculated from the change in concentration per unit time, perfusate volume, and liver weight. Statistical comparisons used Student's *t* test. Values are mean ± standard error.

4-Methylpyrazole	Uptake	
	Ethanol	Methanol
mM	μmol/g/hr	
0	206 ± 18	180 ± 17
0.05	165 ± 9	94 ± 24[a]
0.1	80 ± 45[a]	65 ± 33[a]
1.5	41 ± 20[b]	75 ± 24[a]
5.0	67 ± 21[a]	67 ± 38[a]

[a] $p < 0.05$.
[b] $p < 0.01$.

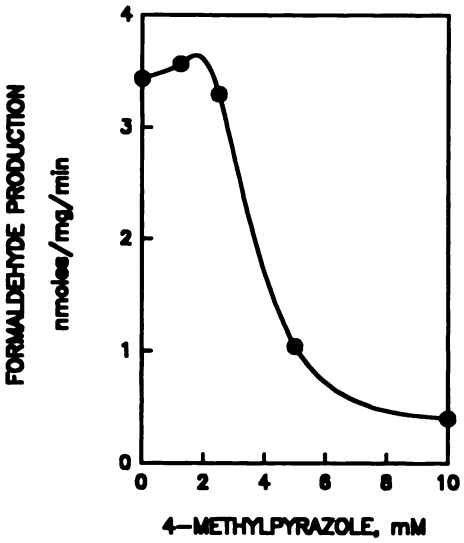

Fig. 2. Rates of formaldehyde production from methanol by liver homogenates from ADH⁻ deer mice. Rates of formaldehyde production from methanol were determined in homogenates as described in Materials and Methods. Typical experiment.

Because significant decreases in catalase-dependent methanol metabolism were observed in the perfused liver, it was important to determine whether 4-methylpyrazole affected the catalase enzyme directly. Catalase activity was 1708 ± 267 units/g of liver in the absence and 1764 ± 459 units/g of liver in the presence of 10 mM 4-methylpyrazole, confirming earlier reports by Feytmans *et al.* (6) that alkyl pyrazoles have no effect on catalase *per se*.

Because catalase was not inhibited yet catalase-dependent methanol metabolism was decreased by 4-methylpyrazole, one possible explanation is that H_2O_2 supply for catalase was diminished by 4-methylpyrazole. This latter possibility was studied by measuring production of formaldehyde from methanol by catalase in liver homogenates, which is dependent on H_2O_2 supply. Indeed, H_2O_2 production was reduced dramatically, by >80%, by 4-methylpyrazole in a dose-dependent manner (Fig. 2).

In order to determine the mechanism for this inhibition, we studied the effect of 4-methylpyrazole on fatty acyl CoA synthetase. This enzyme, located on the outer mitochondrial membrane, peroxisomal membrane, and endoplasmic reticulum, converts fatty acids into acyl CoA compounds (17). Rates of formaldehyde production with various concentrations of palmitate were inhibited competitively by 4-methylpyrazole (Fig. 3A); however, the product palmitoyl CoA had no effect (Fig. 3B). When rates of fatty acyl CoA synthetase were determined directly by measuring the disappearance of CoASH, similar results were obtained (Table 2). These data strongly support the hypothesis that 4-methylpyrazole inhibits H_2O_2 supply for catalase-dependent ethanol metabolism at the level of the fatty acyl CoA synthetase.

If peroxide supply is the limiting step in these studies, treatment with 4-methylpyrazole should also inhibit rates of alcohol metabolism *in vivo* in ADH⁻ deer mice. Methylpyrazole

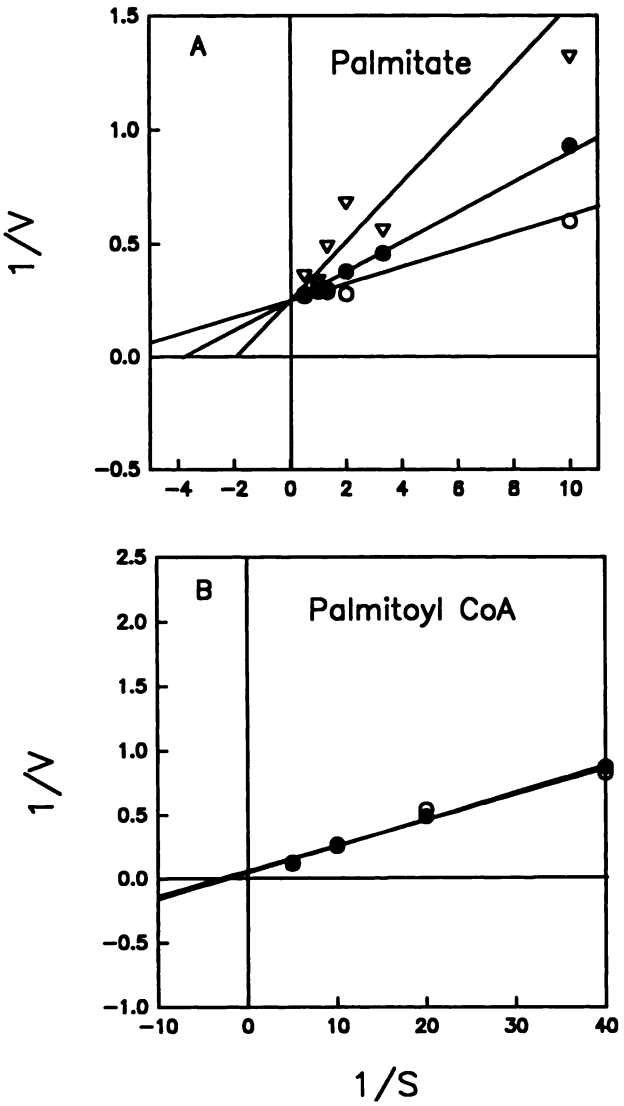

Fig. 3. Effects of 4-methylpyrazole on fatty acyl CoA synthetase. A, Palmitate was varied. Livers from ADH⁻ deer mice were homogenized and assayed for formaldehyde production from methanol, as described in Materials and Methods and Fig. 2. Palmitate (0–2.0 mM) alone (○) or palmitate and 4-methylpyrazole (●, 1 mM; ▽, 5 mM) were incubated with homogenates for 45 min before the addition of reaction mixture, as described in Materials and Methods. B, Palmitoyl CoA was varied. Palmitoyl CoA alone (0–0.2 mM) (○) or palmitoyl CoA and 4-methylpyrazole (5 mM) (●) were added to 1.4 ml of reaction mixture that lacked CoA, as described in Materials and Methods. Homogenate (1/5; 0.1 ml) was added to initiate the reaction and samples were incubated at 37° for 3 min. The reactions were terminated and formaldehyde was determined as described in Materials and Methods.

TABLE 2

Effect of 4-Methylpyrazole on rates of fatty acyl CoA synthetase activity in livers from ADH⁻ deer mice

Rates of fatty acyl CoA synthetase activity were determined in 2000 × *g* supernatants from ADH⁻ deer mouse liver after incubation with 4-methylpyrazole (0–10 mM) as described in Materials and Methods. Rates were measured by determining the disappearance of CoASH over 3 min, as described in Materials and Methods and Ref. 11. Statistical comparisons were performed using Student's *t* test. Values are mean ± standard error for five deer mice.

4-Methylpyrazole	Acyl CoA synthetase
mM	*nmol/mg of protein/min*
0	2008 ± 430
1.25	1883 ± 415
2.5	1860 ± 411
5.0	1714 ± 423[a]
10.0	1682 ± 439[a]

[a] $p < 0.05$.

TABLE 3

Effect of 4-methylpyrazole on rates of ethanol and methanol metabolism in ADH⁺ and ADH⁻ deer mice *in vivo*

Deer mice were given injections of 2 g/kg ethanol or methanol (intraperitoneally) 30 min before treatment with saline or 4-methylpyrazole (MEP) (intraperitoneally) at doses indicated in the table. Alcohol concentrations and rates of alcohol metabolism were determined as described in Materials and Methods for 4–17 deer mice/group. Values are mean ± standard error. Statistical comparisons used Student's *t* test for comparison with saline control.

Strain	Treatment	Metabolism	
		Ethanol	Methanol
		mmol/kg/hr	
ADH⁻	Saline	6.0 ± 0.7	4.6 ± 0.3
	200 mg/kg MEP	3.9 ± 0.7	3.5 ± 0.4
	500 mg/kg MEP	3.9 ± 0.5[a]	2.6 ± 0.5[a]
ADH⁺	Saline	8.0 ± 0.6	4.7 ± 1.0
	200 mg/kg MEP	3.6 ± 0.9[b]	4.3 ± 0.5
	500 mg/kg MEP	3.8 ± 0.4[b]	2.2 ± 0.3[a]

[a] $p < 0.05$.
[b] $p < 0.01$.

(200 mg/kg) tended to decrease both ethanol and methanol metabolism *in vivo* in the ADH⁻ mutant; however, significant decreases of 35–45% were observed with 500 mg/kg methylpyrazole (Table 3). In the ADH⁺ strain, ethanol metabolism was decreased >50% by 200 mg/kg methylpyrazole. Similar results were observed with methanol as the substrate, but 500 mg/kg methylpyrazole was required (Table 3). Thus, the phenomenon described above in the perfused liver also occurs *in vivo*.

Discussion

Effect of 4-methylpyrazole on catalase-dependent alcohol metabolism. The inhibition of alcohol metabolism by 4-methylpyrazole in ADH⁻ deer mice was an unexpected finding. Use of this animal model, which lacks hepatic cytosolic ADH in the liver as well as the selective substrate for catalase (16), methanol, provided a unique opportunity to study the direct effects of 4-methylpyrazole on catalase-dependent alcohol metabolism. Recent work with isotopes has demonstrated that the perfused deer mouse liver is an ideal system to study catalase-dependent alcohol metabolism (18).

In these experiments, 4-methylpyrazole inhibited methanol metabolism by >60% (Table 1). Because methanol is a selective substrate for catalase in rodents and because these experiments were performed using ADH⁻ deer mice, we conclude that 4-methylpyrazole acts directly on the catalase pathway (see Results). This effect must be indirect, however, because Feytmans *et al.* (6) observed, and we confirmed (see Results), that pyrazole and alkyl pyrazoles had no effect on catalase *per se*.

Regulation of catalase-dependent alcohol metabolism by H_2O_2 supply. Catalase-dependent alcohol metabolism has been shown to play a significant role in ADH⁻ deer mice and under some conditions in rats (8, 19). Although older work in perfused liver suggested that catalase played a minor role in alcohol metabolism, those experiments were performed in the absence of fatty acids, the substrate for H_2O_2 generation (19). More recently, Handler and Thurman (20) showed that this pathway could be stimulated significantly by the addition of fatty acids and that the magnitude of the stimulation was dependent upon the chain length of the fatty acid. These findings have been interpreted to indicate that fatty acid metabolism via peroxisomal β-oxidation causes an increase in H_2O_2 supply for catalase-dependent alcohol metabolism. If the supply of H_2O_2 is limiting for catalase-dependent alcohol metabolism in rats and deer mice, then it is reasonable to hypothesize that 4-methylpyrazole acts on catalase-dependent alcohol metabolism by limiting the supply of H_2O_2. In support of this hypothesis, we demonstrated that rates of H_2O_2 formation were inhibited by 4-methylpyrazole in this study (Fig. 2).

Site of action of 4-methylpyrazole. One of the initial steps in fatty acid metabolism occurs in the outer membrane of the mitochondria, the peroxisomal membrane, and the endoplasmic reticulum. There, fatty acyl CoA synthetase (EC 6.2.1.3) converts fatty acids into acyl CoA compounds in reactions that require ATP. It is the CoA form of fatty acids that is transported into the peroxisome for oxidation and production of H_2O_2 (Fig. 4). 4-Methylpyrazole inhibited catalase-dependent alcohol metabolism when the substrate for acyl CoA synthetase, palmitate, but not the product, palmitoyl CoA, was used (Fig. 3; Table 2). Therefore, we conclude that the decrease in ethanol and methanol metabolism described in this study is due to competitive inhibition (see Fig. 3) of fatty acyl CoA synthetase. In this study, alcohol metabolism was inhibited only 50% by 100 μM 4-methylpyrazole in perfused liver (Table 1). Not unexpectedly, higher concentrations were needed *in vivo* and in homogenates, most likely due to nonspecific binding. Theorell and Yonetani (1) designed the alkyl pyrazoles to bind at the lipophilic site on alcohol dehydrogenase. It is logical, therefore, to hypothesize that 4-methylpyrazole also acts at a lipophilic fatty acid binding site, but in this case on fatty acyl CoA synthetase. The results from this study indicate clearly that 4-methylpyrazole is not a specific inhibitor of ADH.

In the 1970s, Blomstrand and colleagues (21, 22) demonstrated that fatty acid metabolism was inhibited by 4-methylpyrazole in the presence of ethanol in human liver slices and *in vivo*. They concluded that 4-methylpyrazole altered the ratio of NADH/NAD⁺, cofactors that are required for ADH-dependent ethanol metabolism. Although this conclusion is plausible, it is also possible that fatty acid metabolism was inhibited at the level of fatty acyl CoA synthetase. In a separate study, Selmer and Grunnet (23) observed that 4-methylpyrazole diminished β-hydroxybutyrate to acetoacetate ratios by >50% in isolated hepatocytes, evidence that further supports the hypothesis that 4-methylpyrazole inhibits fatty acid metabolism. Taken together, these findings are important because 25–40% of fatty acid metabolism occurs via peroxisomal β-oxidation (20, 24).

Has alcohol metabolism via ADH been overestimated?. Surprisingly, we also observed that rates of methanol metabolism were decreased >50% in the presence of ADH *in vivo* (Table 3). Therefore, it is concluded that catalase contributes

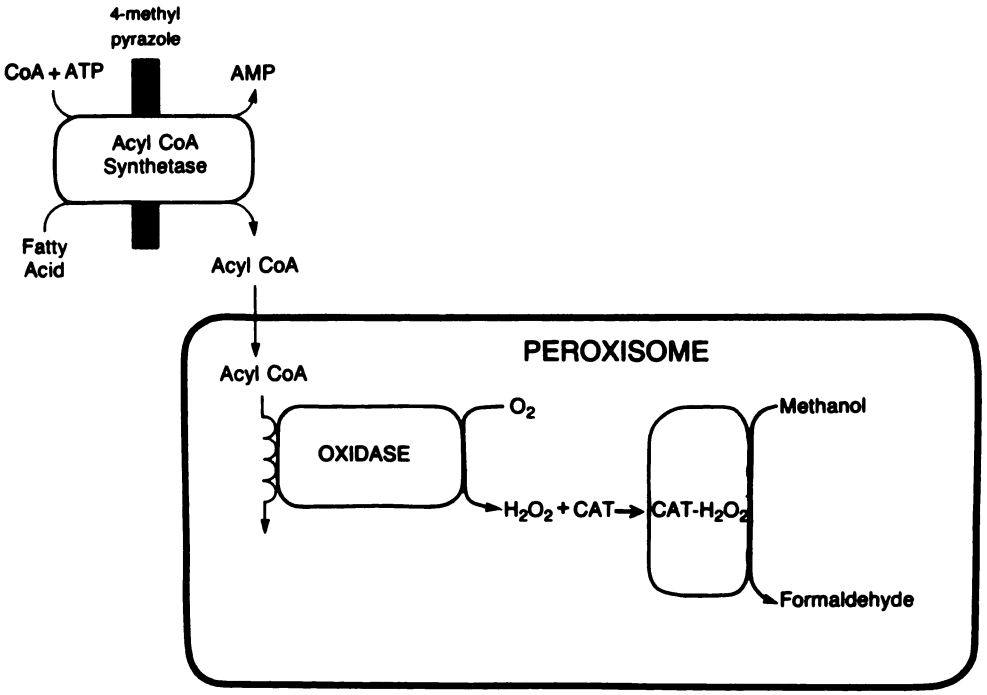

Fig. 4. Scheme depicting the inhibition by 4-methylpyrazole of rates of H_2O_2 production and alcohol metabolism. *CAT*, catalase.

significantly to alcohol metabolism even in the presence of ADH. ADH-dependent metabolism has been claimed to be responsible for >70–80% of total alcohol metabolism; however, these findings suggest that catalase may be more important than ADH. Based on the data in this study, we suggest that, at high concentrations of alcohol, catalase accounts for 60%, ADH 30%, and cytochrome P-450 10% (9) of alcohol metabolism. This calculation is based on the 60% inhibition by 4-methylpyrazole of ethanol and methanol metabolism in perfused liver (Table 2) and estimates in other studies of the contribution of cytochrome P-450 IIE1 (9) of around 10%, with the remainder (i.e., about 30%) being assigned to ADH. Clearly, these data indicate that the contribution of ADH to alcohol metabolism has been previously overestimated.

References

1. Theorell, H., and T. Yonetani. Liver alcohol dehydrogenase-DPN-pyrazole complex: a model of a ternary intermediate in the enzyme reaction. *Biochem. Z.* **338**:537–553 (1963).
2. Lester, D., Z. Keokosky, and F. Felzenberg. Effect of pyrazoles and other compounds on alcohol metabolism. *Quart. J. Stud. Alcohol* **29**:449–454 (1968).
3. Goldberg, L., and U. Rydberg. Inhibition of ethanol metabolism *in vivo* by administration of pyrazole. *Biochem. Pharmacol.* **18**:1749–1762 (1969).
4. Blomstrand, R., and H. Theorell. Inhibitory effect of ethanol oxidation in man after administration of 4-methylpyrazole. *Life Sci.* **9**:631–640 (1970).
5. Damgaard, S. E. The D(V/K) isotope effect of the cytochrome P450-mediated oxidation of ethanol and its biological applications. *Eur. J. Biochem.* **125**:593–603 (1982).
6. Feytmans, E., M. N. Morales, and F. Leighton. Effect of pyrazole on rat liver catalase. *Biochem. Pharmacol.* **23**:1293–1305 (1974).
7. Glassman, E. B., G. A. McLaughlin, D. T. Forman, M. R. Felder, and R. G. Thurman. Role of alcohol dehydrogenase in the swift increase in alcohol metabolism (SIAM). *Biochem. Pharmacol.* **34**:3523–3526 (1985).
8. Handler, J. A., B. U. Bradford, E. B. Glassman, J. K. Ladine, and R. G. Thurman. Catalase-dependent ethanol metabolism *in vivo* in deermice lacking alcohol dehydrogenase. *Biochem. Pharmacol.* **35**:4487–4492 (1986).
9. Handler, J. A., D. R. Koop, M. J. Coon, Y. Takei, and R. G. Thurman. Identification of $P450_{ALC}$ in microsomes from alcohol dehydrogenase-deficient deermice: contribution to ethanol elimination *in vivo*. *Arch. Biochem. Biophys.* **264**:114–124 (1988).
10. Thurman, R. G., H. G. Ley, and R. Scholz. Hepatic microsomal ethanol oxidation, hydrogen peroxide formation and the role of catalase. *Eur. J. Biochem.* **25**:420–430 (1972).
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
12. Inestrosa, N. C., M. Bronfman, and F. Leighton. Detection of peroxisomal fatty acyl-coenzyme A oxidase activity. *Biochem. J.* **182**:779–788 (1979).
13. Nash, T. The colorimetric estimation of formaldehyde by means of the Hantzch reaction. *Biochem. J.* **55**:416–422 (1953).
14. Bar-Tana, J., G. Rose, and B. Shapiro. The purification and properties of microsomal palmitoyl-coenzyme A synthetase. *Biochem. J.* **122**:353–362 (1971).
15. Bergmeyer, H. U. *Methods of Enzymatic Analysis.* Academic Press, New York (1988).
16. Mannering, G. J., D. R. Van Harken, A. B. Makar, T. R. Tephly, W. D. Watkins, and J. I. Goodman. Role of the intracellular distribution of hepatic catalase in the peroxidative oxidation of methanol. *Ann. N. Y. Acad. Sci.* **168**:265–280 (1969).
17. Krisans, S. K., R. M. Mortensen, and P. B. Lazarow. Acyl-CoA synthetase in rat liver peroxisomes: computer-assisted analysis of cell fractionation experiments. *J. Biol. Chem.* **255**:9599–9607 (1980).
18. Cronholm, T., C. Norsten-Hoog, G. Ekstrom, J. A. Handler, R. G. Thurman, and M. Ingelman-Sundberg. Oxidoreduction of butanol in deermice (*Peromyscus maniculatus*) lacking hepatic cytosolic alcohol dehydrogenase. *Eur. J. Biochem.* **204**:353–357 (1992).
19. Oshino, N., D. Jamieson, and B. Chance. The properties of hydrogen peroxide production under hyperoxic and hypoxic conditions of perfused rat liver. *Biochem. J.* **146**:53–65 (1975).
20. Handler, J. A., and R. G. Thurman. Catalase-dependent ethanol oxidation in perfused rat liver: requirement for fatty acid-stimulated H_2O_2 production by peroxisomes. *Eur. J. Biochem.* **176**:477–484 (1988).
21. Blomstrand, R., L. Kager, and O. Lantto. Studies on the ethanol-induced decrease of fatty acid oxidation in rat and human liver slices. *Life Sci.* **13**:1131–1141 (1973).
22. Blomstrand, R., and L. Forsell. Prevention of the acute ethanol-induced fatty liver by 4-methylpyrazole. *Life Sci.* **10**:523–530 (1971).
23. Selmer, J., and N. Grunnet. Ethanol metabolism and lipid synthesis by isolated liver cells from fed rats. *Biochim. Biophys. Acta* **428**:123–137 (1976).
24. Rognstand, R. Estimation of peroxisomal and mitochondrial fatty acid oxidation in rat hepatocytes using tritiated substrates. *Biochem. J.* **279**:147–150 (1991).

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