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METABOLIC ACTIVATION OF *n*-BUTYRALDOXIME BY RAT LIVER MICROSOMAL CYTOCHROME P450

A REQUIREMENT FOR THE INHIBITION OF ALDEHYDE DEHYDROGENASE

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Abstract—*n*-Butyraldoxime (*n*-BO) is known to cause a disulfiram/ethanol-like reaction in humans, a manifestation of the inhibition of hepatic aldehyde dehydrogenase (AlDH). As with a number of other *in vivo* inhibitors of AlDH, *n*-BO does not inhibit purified AlDH *in vitro*, suggesting that a metabolite of *n*-BO is the actual inhibitor of this enzyme. In re-examination of the effect of *n*-BO on blood acetaldehyde levels following ethanol in the Sprague–Dawley rat, we found that pretreatment with substrates and/or inhibitors of cytochrome P450 blocked the *n*-BO-induced rise in blood acetaldehyde in the following order of decreasing potency: 1-benzylimidazole (0.1 mmol/kg) > 3-amino-1,2,4-triazole (1.0 g/kg) > ethanol (3.0 g/kg) > phenobarbital (0.1% in the drinking water, 7 days) > SKF-525A (40 mg/kg). Rat liver microsomes were shown to catalyze the conversion of *n*-BO to an active metabolite that inhibited yeast AlDH. This reaction was dependent on NADPH and molecular oxygen and was inhibited by CO and 1-benzylimidazole. Hydroxylamine, postulated by others to be a metabolite of *n*-BO, inhibited AlDH via a catalase-mediated reaction and not through an NADPH-supported microsome-catalyzed reaction. Using GLC-mass spectrometry, 1-nitrobutane (an N-oxidation product) and butyronitrile (a dehydration product) were identified as metabolites from microsomal incubations of *n*-BO. However, neither of these metabolic products inhibited AlDH directly or in the presence of liver microsomes and NADPH. We conclude that another NADPH-dependent, cytochrome P450-catalyzed metabolic product of *n*-BO is responsible for the inhibition of AlDH by *n*-BO.

Industrial workers exposed to *n*-butyraldoxime (*n*-BO§), an anti-skinning compound used in the manufacture of certain colored inks, experience an alcohol-sensitivity reaction similar to that elicited by disulfiram and other inhibitors of the enzyme aldehyde dehydrogenase (AlDH; EC 1.2.1.3) [1]. Thus, ethanol ingestion following *n*-BO exposure in humans or *n*-BO pretreatment in rats gives rise to elevated blood acetaldehyde levels [1, 2], a consequence of AlDH inhibition. Like pargyline [3], cyanamide [4], coprine [5], and certain other inhibitors of AlDH [6], *n*-BO is a potent inhibitor of this enzyme *in vivo* [2], but not *in vitro* [2, 7], suggesting that a metabolite of *n*-BO is the actual inhibitor of AlDH. The metabolic disposition of *n*-BO has not been described; indeed, there is a paucity of information on the metabolism of aldoximines. On the other hand, ketoximes have been generally considered to be resistant to oxidation by microsomal enzymes and to be stable to other oxidative and/or hydrolytic biotransformations [8, 9].

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§ Abbreviations: ADH, alcohol dehydrogenase; AlDH, aldehyde dehydrogenase; 3-AT, 3-amino-1,2,4-triazole; 1-BI, 1-benzylimidazole; *n*-BO, *n*-butyraldoxime; MS, mass spectrometry; NS, not significant; and PB, phenobarbital.

We have re-examined the effect of *n*-BO on blood acetaldehyde levels following ethanol in rats and found that bioactivation of *n*-BO by the hepatic microsomal cytochrome P450 enzymes is a requirement for the inhibition of AlDH by physiological levels of this drug. Two pathways, viz. oxidative and dehydrative, for the biotransformation of *n*-BO by rat liver microsomes *in vitro* were described recently [10]. These products have been evaluated for their inhibitory properties towards AlDH, but were found to be inactive, thereby implicating yet another metabolite of *n*-BO as the inhibitor of AlDH.

MATERIALS AND METHODS

Materials. Hydroxylamine hydrochloride, 3-amino-1,2,4-triazole (3-AT), bovine liver catalase (C-40), yeast AlDH, glucose, glucose oxidase, $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from the Sigma Chemical Co. (St. Louis, MO). 1-Benzylimidazole (1-BI), 1-nitrobutane and *n*-butyronitrile were obtained from the Aldrich Chemical Co. (Milwaukee, WI), while *n*-BO was from Alfa Products (Ward Hill, MA). *n*-BO was vacuum distilled before use. Reagents for protein determinations were purchased from the Pierce Chemical Co. (Rockford, IL).

Male rats of Sprague–Dawley descent were purchased from Harlan Sprague–Dawley (Indianapolis, IN) and maintained on a certified rat

chow diet (No. 5002; Purina Ralston Co., St. Louis, MO) and water *ad lib.* until used. Dosage forms of ethanol, *n*-BO, SKF-525A and 1-BI were prepared using isotonic saline as vehicle such that 1.0 mL of the injection solutions was administered per 100 g rat body weight. The dosage form of 3-AT was prepared as previously described [11]. The rat liver microsomes were isolated as described earlier [3].

In vivo *studies.* These studies were performed in adherence with guidelines established in the *Guide for the Care and Use of Laboratory Animals* published by the U.S. Department of Health and Human Resources (NIH Publication 85-23, revised 1985). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the research protocol was approved by the Animal Study Subcommittee of the Minneapolis VA Medical Center.

After an overnight fast, the rats (weighing 238 ± 3 g) were naive (not pretreated) (Fig. 1) or pretreated with either SKF-525A, phenobarbital (PB), ethanol, 3-AT, 1-BI or isotonic saline as indicated in Figs. 2 and 3. The animals were administered *n*-BO (i.p.) at zero time followed by ethanol (2 g/kg, i.p.) at 5 or 6 hr where indicated, and were killed 1 hr post-ethanol (or saline) treatment. Blood was collected by open chest cardiac puncture for analysis of acetaldehyde and ethanol as previously described [3].

The rates of ethanol elimination were determined by measuring ethanol levels in duplicate 0.02-mL blood samples obtained from the tail vein at 1, 3 and 5 hr after ethanol administration. The rate of ethanol elimination was calculated as previously described [12].

In vitro *studies.* Rat liver microsomes catalyzed the conversion of *n*-BO to a reactive metabolite that inhibited AlDH. The relative rates of formation of this metabolite were estimated using a two-step assay system with inhibition of yeast AlDH as the end point. The primary reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 5.0 mM $MgCl_2$, 16 mM KCl, 2.5 mM glucose-6-phosphate, 1.0 U glucose-6-phosphate dehydrogenase, and where indicated, *n*-BO, hydroxylamine, nitrobutane or butyronitrile. After a 5-min preincubation period, the reaction was initiated by the addition of $NADP^+$ (0.1 μmol) and 20 μL of uninduced rat liver microsomes (microsomal pellet reconstituted to 0.5 mL/g wet weight liver tissue with 0.1 M potassium phosphate, pH 7.5), followed by yeast AlDH (approximately 0.1 U) 15 sec later. The total volume of the primary reaction mixture was 0.1 mL. This mixture was then incubated for 10 min at 37°. At 10 min, a 20-μL aliquot of the primary mixture was removed and added directly to a secondary reaction mixture containing 0.5 mM NAD^+, 1.0 mM EDTA, 30% glycerol, and 90 mM potassium phosphate buffer (pH 8.0) in a final volume of 1.0 mL. The reaction was initiated by the addition of benzaldehyde (0.6 μmol), and the remaining yeast AlDH activity in this secondary mixture was determined spectrophotometrically by following the increase in absorbance at 340 nm over time.

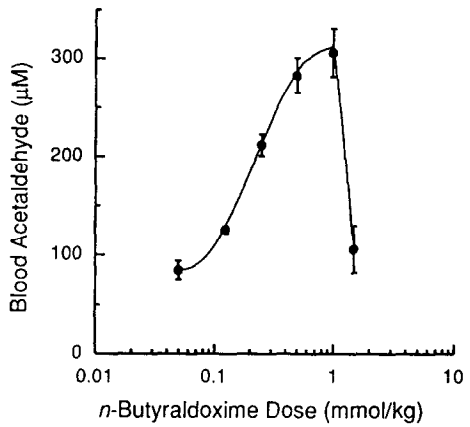

Fig. 1. Log dose versus response curve for the elevation of blood acetaldehyde levels by *n*-BO after ethanol administration to the rat. The animals received *n*-BO (i.p.) followed by ethanol (2 g/kg, i.p.) 6 hr later and were killed 1 hr after ethanol. Each point is the mean ± SEM for three to six animals.

The catalase-mediated bioactivation of hydroxylamine to an inhibitor of yeast AlDH was assessed using the two-step assay system described above, except that the composition of the primary reaction mixture (complete mix) was as follows: 100 mM potassium phosphate buffer (pH 7.5), 16 μg bovine liver catalase, 10 mM glucose, 0.6 U glucose oxidase, 1.0 mM NAD^+, approximately 0.1 U yeast AlDH and hydroxylamine where indicated. The reaction was initiated by the addition of catalase followed by yeast AlDH 30 sec later.

Analytical methods. Blood acetaldehyde and ethanol levels of samples obtained from the heart (Figs. 1–3) were measured by head space gas chromatography as previously described [3]. A different sample preparation procedure was used with blood samples obtained from the tail vein for ethanol analysis (Table 1). Immediately after collection, 20 μL of blood was added to a septum vial containing 20 μL of 50 mM sodium azide, 20 μL of 4.0 mM *n*-propanol (internal standard) and 0.8 g sodium chloride. The samples were sealed and stored on ice until analyzed. Proteins were determined using bicinchoninic acid reagent with bovine serum albumin as the standard [13].

Statistical analysis. The results are expressed as means ± SEM of triplicate samples unless indicated otherwise. Statistical analyses of variance were determined using the Dunnett's test; P values of < 0.05 were accepted as significant.

RESULTS

Inhibition of AlDH in vivo. Measurement of blood acetaldehyde served as a simple screening procedure for following the *in vivo* inhibition of AlDH by *n*-BO in the rat. The ED_{50} for *n*-BO in raising blood acetaldehyde levels was determined to be 0.21 mmol/kg (Fig. 1). Since *n*-BO is an *irreversible* inhibitor of AlDH and a *reversible* inhibitor of alcohol

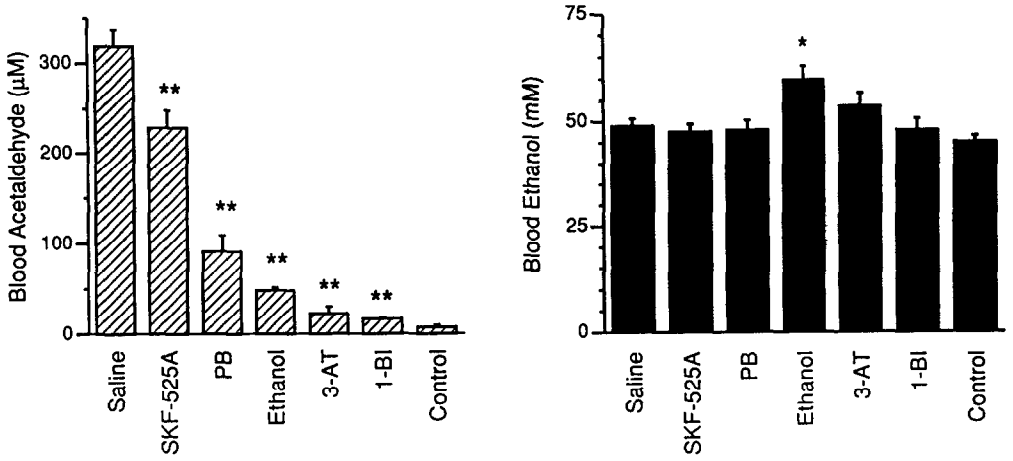

Fig. 2. Effect of pretreatment of rats with saline, SKF-525A, PB, ethanol, 3-AT and 1-BI on the *n*-BO-induced elevation of blood acetaldehyde (left panel) and on blood ethanol levels (right panel) at the time of death. All the animals except the controls received *n*-BO (0.5 mmol/kg, i.p.) followed by ethanol (2.0 g/kg, i.p.) 5 hr later and were killed 1 hr after ethanol. Controls were administered only saline and ethanol. In the pretreated groups, saline was given 1 hr before *n*-BO; SKF-525A (40 mg/kg, i.p.) was given 30 min before *n*-BO; PB was given in the drinking water as a 0.1% solution for 7 days; 3-AT (1.0 g/kg, i.p.) was administered 3 hr before *n*-BO, and 1-BI (0.1 mmol/kg, i.p.) was given 30 min before *n*-BO. Those animals pretreated with ethanol were administered ethanol (3.0 g/kg, i.p.) 1 hr before *n*-BO and saline 5 hr after *n*-BO, and were killed 1 hr after saline. Each value is the mean ± SEM for four to six animals. P values of < 0.05 (*) and < 0.01 (**) vs saline-pretreated animals are indicated.

dehydrogenase (ADH) [2], it was necessary to prolong the time interval between the administration of *n*-BO and the administration of ethanol in order to provide sufficient time for the *n*-BO to clear, thereby allowing the ethanol to be oxidized to acetaldehyde. Inhibition of ADH by *n*-BO would interfere with the expected rise in blood acetaldehyde following ethanol administration. The sharp drop in blood acetaldehyde levels when the *n*-BO dose was > 1.0 mmol/kg (Fig. 1) is interpreted to reflect this (reversible) inhibition of ADH.

The possible involvement of either microsomal cytochrome P450 or catalase in the bioactivation of *n*-BO in the rat was assessed by determining the effect of pretreatment with a variety of substrates and inhibitors of these enzymes on *n*-BO-induced acetaldehydemia in the rat (Fig. 2). SKF-525A, a competitive inhibitor of cytochrome P450 [14], caused only a modest reduction in ethanol-derived blood acetaldehyde levels, whereas 1-BI, a ligand-binding inhibitor of cytochrome P450 [15, 16], completely blocked the rise in *n*-BO-induced blood acetaldehyde. PB, ethanol and 3-AT pretreatments also caused significant reductions in blood acetaldehyde levels.

The dose–effect relationship of 1-BI to inhibition of AlDH *in vivo* as reflected by decreased acetaldehydemia for a constant 0.5 mmol/kg dose of *n*-BO is shown in Fig. 3. The ED_{50} was found to be 6.0 μmol/kg.

Since inhibition of *ethanol* oxidation by PB, 3-AT or 1-BI could also cause decreased acetaldehydemia, the effects of these substances on *in vivo* ethanol elimination were compared in rats treated with *n*-BO (Table 1). *n*-BO (0.5 mmol/kg) alone reduced

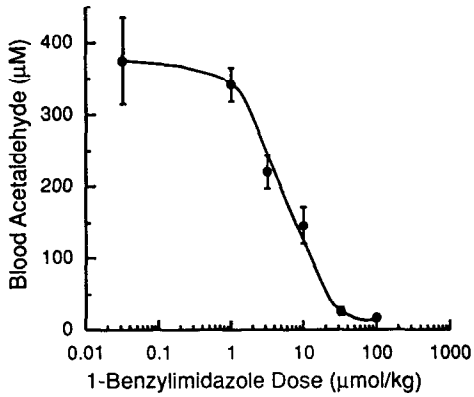

Fig. 3. Log dose versus response curve for the effect of 1-BI on *n*-BO-induced acetaldehydemia in the rat. Using the protocol described in Fig. 2, the dose of 1-BI was varied. Each value is the mean ± SEM for three to five animals.

the rate of ethanol elimination by 40% relative to saline controls, whereas *n*-BO administered to PB-pretreated rats did not reduce significantly the rate of ethanol metabolism. The rates of ethanol elimination in 3-AT plus *n*-BO-treated rats were similar (P, NS) to rats treated with *n*-BO alone. In contrast, 1-BI administered with or without *n*-BO significantly reduced the rate of ethanol elimination. The rate of ethanol elimination in 1-BI plus *n*-BO treatment was reduced significantly compared with those treated with *n*-BO alone ($P < 0.01$). From these results we conclude that inhibition of ethanol

Table 1. Effect of pretreatments on *in vivo* ethanol elimination in the rat*

Pretreatments	N	Rate of ethanol elimination (mmol/kg body wt per hr)	Rate of ethanol elimination (% of control)
Control	3	6.85 ± 0.53	100
n-BO (0.5 mmol/kg)	7	3.97 ± 0.29†	58
PB, *n*-BO	3	6.22 ± 0.20	91
3-AT (1.0 g/kg), *n*-BO	3	4.62 ± 0.39†	67
1-BI (0.03 mmol/kg), *n*-BO	3	1.84 ± 0.17†	27
1-BI (0.1 mmol/kg), *n*-BO	3	<1.0†	<15
1-BI (0.03 mmol/kg)	3	3.93 ± 0.71†	57
1-BI (0.1 mmol/kg)	3	2.66 ± 0.07†	39

* The animal protocol and drug dosages other than those indicated above were as described in the legend of Fig. 2. Results are means ± SEM.
† P value of < 0.01 vs the saline control.

oxidation may have contributed to the reduction of blood acetaldehyde levels in 1-BI-pretreated rats, but not in PB- or 3-AT-pretreated rats.

Inhibition of AlDH by hydroxylamine. Koe and Tenen [2] suggested that hydroxylamine, a hydrolysis product of *n*-BO, might be the metabolite responsible for the inhibition of AlDH *in vivo*. Using yeast AlDH as a model for the mammalian enzyme,* hydroxylamine was found *not* to inhibit yeast AlDH activity *directly*; however, it inhibited this enzyme by approximately 80% in the presence of catalase and a hydrogen peroxide-generating system of glucose/glucose oxidase (Fig. 4). This catalase/hydrogen peroxide-mediated inhibition of AlDH was independent of NAD^+. Using the same assay conditions described in Fig. 4, the IC_{50} for hydroxylamine as an inhibitor of yeast AlDH was found to be 52 μM (data not shown). The approximately 20% inhibition of AlDH by hydroxylamine plus hydrogen peroxide in the absence of catalase (Fig. 4) was due to a direct effect of the latter on AlDH activity.

Since pretreatment of the rats with 3-AT, an inhibitor of catalase [19], or ethanol, a substrate for catalase, significantly attenuated the *n*-BO-induced rise in blood acetaldehyde (Fig. 2), the possibility was considered that catalase might be the enzyme responsible for the bioactivation of *n*-BO. However, catalase did *not* convert *n*-BO to an active inhibitor, as evidenced by the failure of *n*-BO to inhibit AlDH in the presence of catalase and a hydrogen peroxide-generating system (Table 2). In contrast, hydroxylamine was bioactivated by catalase/hydrogen peroxide to a potent inhibitor of AlDH, corroborating the earlier result (Fig. 4).

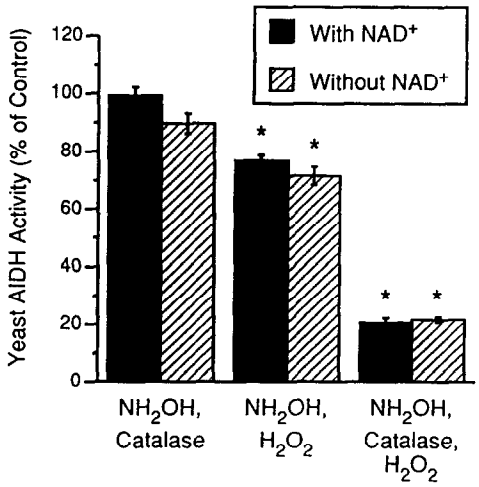

Fig. 4. Inhibition of yeast AlDH by hydroxylamine (NH_2OH) mediated by catalase/hydrogen peroxide (H_2O_2) with and without NAD^+. Yeast AlDH was preincubated with hydroxylamine in a primary reaction mix in the presence of catalase and a hydrogen peroxide-generating system (glucose/glucose oxidase) where indicated (H_2O_2). The AlDH activity remaining after this preincubation period was measured using a secondary reaction mix as described under Materials and Methods. Yeast AlDH activity was equal to 16.5 ± 0.8 nmol NAD^+ reduced/min in the controls without hydroxylamine. Results are the means ± SEM for three to four incubations. P values of < 0.01 (*) vs control are indicated.

Metabolic activation of n-BO by hepatic microsomal cytochrome P450. Isolated rat liver microsomes, when incubated in the presence of an NADPH-generating system, catalyzed the conversion of *n*-BO to an active form that inhibited yeast AlDH (Fig. 5). The IC_{50} for *n*-BO in this cytochrome P450/NADPH catalyzed reaction was 38 μM (data not shown). Rat liver microsomal preparations contain significant catalase activity [20] and since catalase can convert hydroxylamine to an inhibitor of AlDH (Table 2), the inhibitory activities of hydroxylamine and *n*-BO toward yeast AlDH were compared using

* Our rationale for using yeast AlDH as a model for the mammalian mitochondrial AlDH isozyme is based on their similar kinetic properties and reaction mechanisms [17] as well as the conservation of key amino acid residues believed essential for activity [18]. In addition, yeast AlDH which is 40–200 times more active than purified mammalian liver isozyme [17] is commercially available and spares the use of experimental animals for enzyme isolation. Substances that inhibit yeast AlDH have been shown to inhibit the low K_m mammalian AlDH, and vice versa [3–5].

Table 2. Inhibition of yeast AlDH activity mediated by the action of bovine liver catalase on hydroxylamine and *n*-BO*

Inhibitor	Yeast AlDH activity (% of control)	
	Control	Catalase/H_2O_2
None	100.0 ± 1.8	101.8 ± 3.5
Hydroxylamine (1.0 mM)	92.4 ± 4.1	5.0 ± 1.1
n-BO (1.0 mM)	93.6 ± 5.3	95.4 ± 4.1

* Yeast AlDH was preincubated with inhibitor in a primary reaction mix in the presence or absence (control) of catalase/H_2O_2, the latter supplied by a generating system of glucose/glucose oxidase. The AlDH activity remaining after incubation of the primary mix was measured using a secondary reaction mix as described in Materials and Methods. Control yeast AlDH activity was equal to 17.1 ± 0.3 nmol NAD^+ reduced/min. Results are the means ± SEM for three incubations.

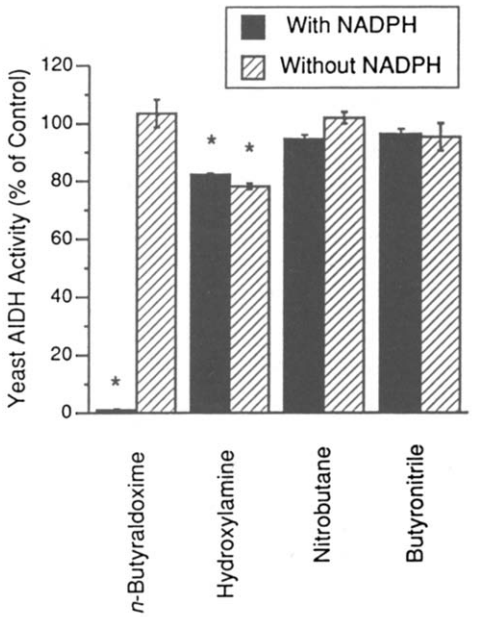

Fig. 5. Inhibition of yeast AlDH by *n*-BO, hydroxylamine, 1-nitrobutane and butyronitrile mediated by uninduced rat liver microsomes with and without NADPH. NADPH was generated by the addition of $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase. Other details were as described under Materials and Methods. The activity of yeast AlDH was 17.0 ± 0.7 nmol NAD^+ reduced/min in controls without inhibitor. Results are the means ± SEM for three to five incubations. P values of < 0.01 (*) vs control are indicated.

the microsomal cytochrome P450 system for bioactivation (Fig. 5). In the presence of NADPH, *n*-BO completely inhibited yeast AlDH, whereas hydroxylamine at equimolar concentrations inhibited this enzyme by only 20% in the presence or absence

Table 3. Effect of N_2 atmosphere, CO and 1-BI on the inhibition of yeast AlDH mediated by the oxidation of *n*-BO by rat liver microsomes*

Additions	Atmosphere	Yeast AlDH activity (% of control)
None	Air	100.0 ± 1.6
n-BO (1.0 mM)	Air	7.8 ± 0.4
n-BO	N_2†	83.4 ± 3.9
n-BO	80:20 CO:O_2	50.7 ± 5.1
n-BO, 1-BI (1.0 mM)	Air	84.8 ± 0.6

* Yeast AlDH was preincubated with *n*-BO (and 1-BI) in a primary reaction mix under an atmosphere of air, N_2 or CO/O_2. AlDH activity remaining after preincubation was measured using a secondary reaction mix as described in Materials and Methods. Control yeast AlDH activity was equal to 32.7 ± 0.5 nmol NAD^+ reduced/min. Results are the means ± SEM for three incubations.

† Anaerobic conditions were achieved by flushing the head space of the primary reaction mix with N_2 gas for 3 min and including glucose (10 mM), glucose oxidase (140 U) and catalase (65 μg) to remove the last traces of O_2 [21].

of NADPH. If hydroxylamine were an intermediate in the conversion of *n*-BO to its active form, it should have inhibited AlDH completely under these conditions. We conclude that hydroxylamine is *not* involved in the cytochrome P450-catalyzed conversion of *n*-BO to an active metabolite that inhibits AlDH.

Further evidence for a direct role of cytochrome P450 in the bioactivation of *n*-BO to an inhibitor of AlDH was provided by examining the effect of anaerobic conditions and two ligand binding inhibitors of cytochrome P450, i.e. CO and 1-BI, on this reaction. The metabolic conversion of *n*-BO to an inhibitor of AlDH by hepatic microsomes, as assessed by their effect on AlDH activity, was highly dependent on the presence of oxygen and was inhibited by CO and 1-BI (Table 3). The degree of AlDH inhibition in anaerobic incubations or in the presence of CO or 1-BI paralleled their inhibitory effect on microsomal cytochrome P450-catalyzed oxidative metabolism of *n*-BO [10].

Evaluation of 1-*nitrobutane and* n-*butyronitrile, end-products of* n-*BO metabolism, as possible inhibitors of AlDH.* As little was known about the metabolism of oximes in general and the metabolism of aldoximes in particular, we examined the volatile end-products formed when *n*-BO was incubated with PB-induced rat liver microsomes *in vitro*. Two major GLC peaks were observed. These volatile metabolites of *n*-BO were identified by GLC-mass spectrometry (MS) to be 1-nitrobutane and *n*-butyronitrile [10]. When these metabolites were incubated with yeast AlDH alone or in the presence of rat liver microsomes and NADPH (Fig. 5), neither nitrobutane (1.0 mM) nor butyronitrile (1.0 mM) inhibited this enzyme. The above results indicate that these metabolites are not direct inhibitors of AlDH and are also not precursors of the active metabolite. Cytochrome

P450 must, therefore, metabolize *n*-BO to its active form by way of a third, as yet undiscovered, reaction pathway.

DISCUSSION

The significant increase in blood acetaldehyde levels observed after ethanol administration to *n*-BO-treated rats (Fig. 1) and the lack of direct inhibition of purified yeast AlDH by *n*-BO (Table 2) confirm earlier reports [1, 2, 7] that *n*-BO is a potent inhibitor of AlDH *in vivo*, but is ineffective *in vitro*. These results indicate that a *metabolite* of *n*-BO is the actual inhibitory species and not the parent compound.

Koe and Tenen [2] suggested that hydroxylamine was a likely metabolite of *n*-BO and might perhaps be the actual inhibitory species. We found that hydroxylamine was indeed a potent inhibitor of AlDH when incubated *in vitro* with catalase and a hydrogen peroxide source (Fig. 3), the IC_{50} for this reaction being 52 μM. Furthermore, ethanol, a substrate for catalase, and 3-AT, an inhibitor of catalase [19], significantly reduced *n*-BO-induced acetaldehydemia in animals pretreated with these agents (Fig. 2), suggesting the possible involvement of catalase in the bioactivation of *n*-BO. However, when *n*-BO was incubated in this *in vitro* system of catalase/hydrogen peroxide source, no inhibition of AlDH was observed (Table 2). Nevertheless, these data do not rule out the possibility that *n*-BO might be hydrolytically cleaved to *n*-butyraldehyde and hydroxylamine *in vivo*, and the latter, in turn, converted to the active inhibitor by AlDH.

Isolated rat liver microsomes were found to convert *n*-BO to an active form that inhibited AlDH through an NADPH-dependent process (Fig. 5). As microsomal preparations are known to be contaminated with catalase [20], it was possible that any hydroxylamine formed in this system could be converted to an inhibitory species by catalase in the presence of hydrogen peroxide. However, at equimolar concentration with *n*-BO and under conditions where *n*-BO completely inhibited AlDH, hydroxylamine had only a modest effect on AlDH activity in the presence of liver microsomes (Fig. 5). From these results, we conclude that hydroxylamine is not an intermediate in the conversion of *n*-BO to its active form.

The *in vivo* inhibition studies shown in Fig. 2 can be interpreted in support of a role for microsomal cytochrome P450 in the bioactivation of *n*-BO. Of the two inhibitors of cytochrome P450 tested, viz. SKF-525A [14] and 1-BI [15, 16], only the latter markedly blocked the rise in acetaldehyde blood levels induced by *n*-BO. The differential effects of these inhibitors may be attributed to the differential inhibition of isoforms of cytochrome P450 by SKF-525A. The effect of 3-AT on *n*-BO-induced blood acetaldehyde levels may also be due to a direct inhibition of cytochrome P450 by 3-AT [22, 23]. Although ethanol is a substrate for cytochrome P450IIE1 and may partially block the bioactivation of *n*-BO by competitive substrate inhibition, it is also possible that ethanol-derived acetaldehyde protects hepatic AlDH by binding to the active site sulfhydryl group of AlDH. PB, an inducer of cytochrome P450IIB1, caused a reduction in blood acetaldehyde levels rather than the expected increase associated with cytochrome P450 induction. This may be due to: (a) a concomitant induction of the cytosolic high K_m AlDH isozymes by PB [24, 25], thus allowing more acetaldehyde to be metabolized or (b) PB-induced cytochrome P450 isoforms may metabolize *n*-BO preferentially to innocuous products, i.e. nitrobutane and butyronitrile (as discussed below), rather than the inhibitory species.

Since the rate of ethanol metabolism may also influence the levels of blood acetaldehyde, the effects of PB, 3-AT and 1-BI on the rate of ethanol elimination were studied. *n*-BO given alone reduced the rate of ethanol elimination by 40% (Table 1). This decrease in rate of ethanol metabolism may be due to reversible inhibition of ADH by unmetabolized *n*-BO still present at 5 hr, or to the inhibitory effect of a high concentration of acetaldehyde on the ADH reaction [26]. PB or 3-AT pretreatment followed by *n*-BO did not cause a further reduction in the rate of ethanol elimination (Table 1). In fact, the rate of ethanol elimination in PB-pretreated rats given *n*-BO was equal to the rate in saline controls (Table 1). However, 1-BI given alone significantly reduced the rate of ethanol elimination in rats and 1-BI plus *n*-BO reduced the rate more than *n*-BO alone (Table 1). Therefore, the reduction of *n*-BO-induced blood acetaldehydemia in 1-BI-pretreated rats may be due, in part, to a decrease in the rate of ethanol elimination.

In vitro studies provide further support for a role for cytochrome P450 in the bioactivation of *n*-BO. *n*-BO was effectively converted to an active inhibitor of AlDH by isolated rat liver microsomes through an NADPH-supported reaction (Fig. 5), the IC_{50} being 38 μM for this reaction. The formation of the *n*-BO-derived inhibitor was also dependent on molecular oxygen (Table 3). In addition, two ligand binding inhibitors of cytochrome P450, viz. CO and 1-BI [15, 16], blocked the conversion of *n*-BO to an inhibitory metabolite (Table 3). The requirements of NADPH and molecular oxygen for microsomal catalyzed *n*-BO bioactivation and the inhibition of this reaction by known inhibitors of cytochrome P450 are consistent with an oxidative biotransformation of *n*-BO by cytochrome P450.

Little is known regarding the metabolism of aldoximes. Since ketoximes are considered to be resistant to oxidative and hydrolytic biotransformations [8, 9; however, *vide infra*] and the metabolic fate of aldoximes was unknown, there was no known precedent for the types of metabolites to expect. We, therefore, used a combination of GLC and GLC/MS techniques to identify the volatile products produced upon incubation of *n*-BO with rat liver microsomes. Using these methods, two substances were identified as stable end-products of *n*-BO metabolism, namely, 1-nitrobutane and *n*-butyronitrile [10].

1-Nitrobutane, formed by direct oxygenation of the oximino nitrogen of *n*-BO, was an expected metabolite. For example, 2-nitro-1-phenylpropane [27] has been identified as a metabolite of 1-phenyl-2-propane oxime and 2-nitropropane [28] as a

metabolite of acetoxime (2-propanone oxime), both reactions being catalyzed by rat liver microsomes. The formation of butyronitrile, a dehydration product of *n*-BO, represents a unique cytochrome P450 catalyzed reaction [10]. The formation of these metabolites from *n*-BO was dependent on the presence of NADPH and was effectively inhibited by CO and 1-BI, two ligand-binding inhibitors of cytochrome P450 [10].

However, neither of these *in vitro* metabolites of *n*-BO was a direct inhibitor of yeast AlDH. They were also not metabolized to an AlDH inhibitor by microsomal enzymes of rat liver (Table 2). Therefore, a third as yet undiscovered NADPH-dependent microsomal reaction that leads to the formation of an inhibitor of AlDH must exist. This third, albeit speculative, reaction may involve cytochrome P450-catalyzed oxidative metabolism of *n*-BO to produce nitroxyl (HNO), a known inhibitor of AlDH [29, 30]. Studies are in progress to evaluate this possibility.

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