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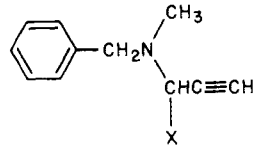
Communications to the Editor

Propiolaldehyde, a Pargyline Metabolite That Irreversibly Inhibits Aldehyde Dehydrogenase. Isolation from a Hepatic Microsomal System

Sir:

The characteristic "disulfiram syndrome" associated with certain alcohol–drug interactions[1] has been attributed in part to acetaldehydemia and subsequent release of biogenic amines.[2] The ability of pargyline (*N*-methyl-*N*-propargylbenzylamine, 1) to cause marked elevations



1, X = H
2, X = OH

in ethanol-derived blood acetaldehyde (AcH) levels in rodents in a manner similar to that observed with disulfiram (Antabuse®) is well documented.[3,4] Unlike disulfiram, however, 1 is not an effective inhibitor of aldehyde dehydrogenase (AlDH) in vitro, suggesting that a metabolite of 1 is the active inhibitory species in vivo. Formation of this inhibitor in vivo appears to be mediated by the hepatic cytochrome P-450 enzymes, as evidenced by the ability of SKF-525A to nullify the pargyline-induced rise in blood AcH after ethanol[4] and by the nearly twofold *enhancement* of this effect by phenobarbital pretreatment (Figure 1).

Indirect evidence from our laboratory suggested that this active pargyline-derived inhibitor of AlDH is propiolaldehyde (HC≡CCHO).[4] We have now succeeded in trapping propiolaldehyde as its semicarbazone by incubating 1 with a phenobarbital-induced rat liver microsomal preparation. Isolation of the semicarbazone, characterization by reference to an authentic sample on TLC, and subsequent release of propiolaldehyde and identification of the latter by its gas chromatographic (GC) retention time and by gas chromatography–mass spectrometry (GC–MS) constitute unequivocal proof that this acetylenic aldehyde is a metabolite of 1. We also present evidence that the propiolaldehyde inhibition of mitochondrial AlDH is irreversible.

Propiolaldehyde semicarbazone was prepared from a synthetic sample of propiolaldehyde[5] and recrystallized from absolute EtOH (or benzene for small samples): mp 169–170 °C with dec (lit.[6] 165–166 °C); UV λ_{max} 261 nm (ϵ 15000, MeOH); IR (KBr) 3420, 3230, 3170 (s, N-H), 3350 or 3310 (m, ≡CH), 3050 (w, –CH=), 2080 (m, C≡C), 1730 (s, C=O), 1615 (s, NH_2 deformation), 1460 (s) cm^{-1}; NMR (Me_2SO-d_6, Me_4Si) δ 9.25 (s, 1 H, NH), 6.56 (d, J = 1.5 Hz, 3 H, CH=N, NH_2), 4.96 (d, J = 1.5 Hz, 1 H, ≡CH[7]). Anal. ($C_4H_5N_3O$) C, H, N (reported:[6] N only).

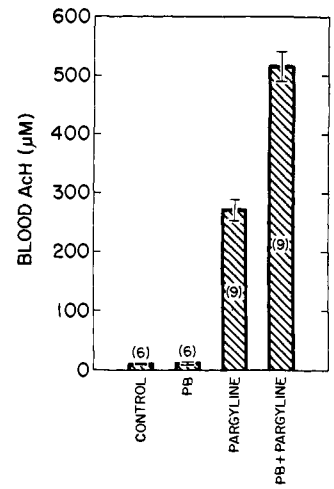


Figure 1. Enhancement of ethanol-derived blood AcH by administration of pargyline (1) to phenobarbital-treated rats. Male Sprague–Dawley rats were maintained ad libitum on chow and 0.1% phenobarbital in the drinking water for 8 days but were fasted overnight just before the experimental day. Blood AcH levels were measured 2 h after 1 (1.25 mmol/kg, ip) and 1 h after an acute ethanol dose (2.0 g/kg, ip). Means ± SE; n values in parentheses; p value (Student's t test) for PB + pargyline group vs. pargyline alone was <0.001.

Pargyline hydrochloride (1·HCl, 3 mM) was incubated at 37 °C for 30 min in each of six flasks containing sodium phosphate buffer (pH 7.4, 83 mM), $NADP^+$ (2.0 mM), glucose 6-phosphate (2.5 mM), KCl (16.5 mM), $MgCl_2$ (4.0 mM), nicotinamide (8.3 mM), semicarbazide hydrochloride adjusted to pH 7.4 (30 mM), and the 9000*g* supernatant fraction from phenobarbital-pretreated rat liver homogenates (500 mg wet wt of liver) in a final volume of 12 mL. Glucose 6-phosphate and $NADP^+$ were omitted from control incubations. The reaction was initiated by the addition of enzyme and terminated by dilution of the system with 15 mL of cold 0.1 M phosphate buffer, pH 7.4. Propiolaldehyde semicarbazone was extracted from the incubation mixture with 5 × 200 mL of EtOAc. The residue remaining after evaporation of the solvent was extracted with hot benzene, and this benzene extract was subjected to column chromatography on silica gel using a benzene–EtOAc gradient. Fractions containing a component with R_f values identical to authentic propiolaldehyde semicarbazone were combined and acidified with 5 N $HClO_4$ to release propiolaldehyde, which was identified by a head space gas chromatographic procedure identical to that previously described for AcH.[8] Synthetic propiolaldehyde and propiolaldehyde semicarbazone were used as standards. Control samples where cofactors were

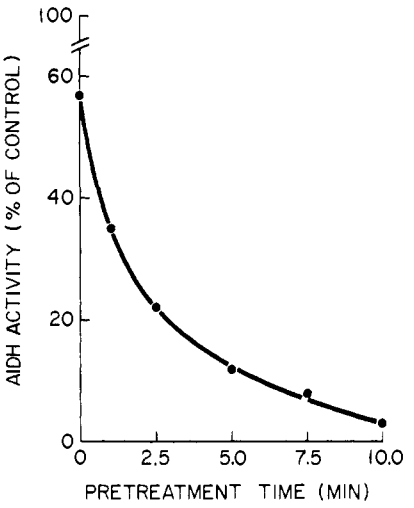


Figure 2. Irreversible inhibition of mitochondrial AlDH by propiolaldehyde. Rat liver mitochondria (11.9 mg/mL protein) were isolated (0.25 M sucrose–0.1 mM EDTA, pH 7.5) from male Sprague–Dawley rats. The mitochondria were pretreated over the times indicated at 38 °C with or without (control) 1.0 mM propiolaldehyde in a reaction mixture containing 0.25 M sucrose, 3.0 μmol of $MgSO_4$, 0.6 μmol of EDTA, and 6.0 μmol of arsenate (pH 7.5), in a final volume of 2.0 mL. Following pretreatment, the mitochondria were immediately chilled in ice and washed three times with 0.25 M sucrose, and the AlDH activity was determined.[4] Each point plotted represents the mean of triplicate analyses. The 100% AlDH activity corresponded to 9.98 ± 0.45 (SE) nmol of AcH oxidized min^{-1} (mg of protein)$^{-1}$. No inhibition was observed over this time period in the absence of propiolaldehyde.

omitted were processed similarly. Only those samples derived from extracts of the complete incubation system released a volatile component with a GC retention time (214 ± 2 s) identical to authentic propiolaldehyde.

Propiolaldehyde was also detected, but in lesser amounts, when **1** was incubated with rat liver preparations from uninduced rats. However, for convenience in isolation, phenobarbital-treated rats were routinely used to increase the levels of the acetylenic aldehyde formed. The results were essentially identical when liver microsomes (100000*g* fraction) from phenobarbital-treated rats were incubated with **1** in the presence of an NADPH-generating system. GC–MS analysis (Tenax-GC, 35–60 mesh, 122-cm glass column, 100 °C isothermal, 290 °C ion-source temperature, LKB 9000 GC–mass spectrometer) of this pargyline-derived propiolaldehyde at 20 and/or 70 eV gave ions at *m/e* 54 ($M^{+\cdot}$), 53 $(M - 1)^+$, and 26 $(C_2H_2)^{+\cdot}$ characteristic for and identical with that from authentic propiolaldehyde.

Mitochondrial AlDH activity was assayed following pretreatment of intact rat liver mitochondria with propiolaldehyde (1.0 mM) at 38 °C for short periods followed by immediate washing (three times) with 0.25 M sucrose. Appreciable inhibition occurred even on momentary contact of propiolaldehyde with mitochondria amounting to 43% inhibition at "zero" time. Inhibition was nearly complete after 5 min and was complete after 10 min of pretreatment (Figure 2). At lower temperatures, viz., 25 and 0 °C, the degrees of inhibition measured after 5 min of pretreatment with propiolaldehyde were correspondingly decreased to 45 and 32% of controls, respectively. The temperature and contact-time dependence of inhibition of mitochondrial AlDH by propiolaldehyde and the fact that the enzymatic activity could not be restored by repeated washing of the mitochondria suggest that this inhibitor is binding covalently to the enzyme. This strong inhibition by propiolaldehyde of AlDH—an NAD^+-linked enzyme—appears to differ mechanistically from the inhibition of the FAD-linked enzymes, monoamine oxidase, and *N,N*-dimethylglycine oxidase, since propiolaldehyde has been shown not to appreciably inhibit the latter.[9]

The oxidative N-depropargylation of **1** to propiolaldehyde, a phase I drug biotransformation reaction involving an acetylenic (propargyl) side chain, was predictable on the basis of our previous studies[4] and the observations of Diehl et al.[10] that *N*-methylbenzylamine was a major urinary metabolite of **1**, with *N*-benzylpropargylamine, the product of oxidative N-demethylation, constituting the other major urinary metabolite. The likely intermediate for this cytochrome P-450 catalyzed N-depropargylation reaction being the carbinolamine **2**, the present results further support the tenet that a triple-bonded carbon in a xenobiotic substance, including that of an aliphatic nitrile, is not oxidized directly but is metabolized at a position α to this triple bond.[11]

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