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PREFERENTIAL INHIBITION OF THE LOW K_m ALDEHYDE DEHYDROGENASE ACTIVITY BY PARGYLINE*

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Abstract—Pargyline has previously been shown to increase ethanol-induced sleep time, decrease ethanol elimination rate and greatly increase acetaldehyde levels after ethanol administration in mice. In rats pargyline treatment did not significantly alter blood ethanol levels but increased blood acetaldehyde levels in a dose-dependent manner. Using 5 mM propionaldehyde to assay aldehyde dehydrogenase activity only about 40 per cent inhibition of the mitochondrial aldehyde oxidizing capacity was seen with the highest pargyline dose (100 mg/kg). Almost total inhibition of the low K_m mitochondrial aldehyde dehydrogenase activity was observed with 50 μM propionaldehyde or 1 mM formaldehyde.

Administration of 40 mg/kg of the monoamine oxidase inhibitor pargyline to mice has been shown to decrease ethanol elimination by approximately 40 per cent and to cause a 3-fold increase in sleep time [1]. Recently ethanol elimination rate decreases of about 40 per cent have been reported after administration of 100 mg/kg of pargyline [2]. Blood acetaldehyde levels, however, increased 15- to 20-fold in these same mice. Cohen *et al.* [3] previously reported 20-fold increases in blood acetaldehyde levels in mice and 12-fold increases in rats given 100 mg/kg of pargyline.

Dembiec, MacNamee and Cohen [2] also reported 58 per cent inhibition by 100 mg/kg pargyline of aldehyde dehydrogenase (AlDH, EC 1.2.1.3, Aldehyde: NAD oxidoreductase) activity measured in liver homogenates. Diethyldithiocarbamate inhibited AlDH more than pargyline but caused elevation of blood acetaldehyde to levels only about one-half as high a level as those produced by pargyline administration. It is, therefore, quite possible that the correlation between increases of the blood acetaldehyde levels and the inhibition of the activity of one AlDH enzyme may be more relevant than total AlDH activity.

In preliminary experiments in rats we used millimolar concentrations of propionaldehyde or acetaldehyde to assess AlDH activity in total liver homogenates or supernatant fractions. Only slight inhibition occurred after administration of near-lethal pargyline doses. In rats, acetaldehyde produced during ethanol metabolism is oxidized principally in a liver compartment other than the cytosol, presumably in the mitochondria [4]. Acetaldehyde is oxidized almost exclusively in the mitochondria when arterial-venous aldehyde concentration is below 0.4 mM [5]. This level is equivalent to 20 μg/ml of acetaldehyde and corresponds to maximum levels encountered even after administration of high pargyline doses.

Marchner and Tottmar [6] suggest that the high K_m hepatic AlDH's have only minor importance in acetaldehyde oxidation. They suggest that the low K_m mitochondrial AlDH, the mitochondrial enzyme I, plays the major role in acetaldehyde metabolism *in vivo*. Since cytosolic and microsomal AlDH activities have little importance in acetaldehyde metabolism under normal conditions [7, 8], pargyline probably does not cause increased acetaldehyde levels through inhibition of these enzymes.

Two recent papers report partial purification of two mitochondrial AlDH's which differ in submitochondrial localization, K_m for aldehydes, K_m for NAD and substrate specificity [7, 8]. At a propionaldehyde concentration of 0.05 mM, little oxidation by the high K_m AlDH would be expected since its K_m for propionaldehyde is 0.45 [7] or 0.5 mM [8]. These studies have also shown that the low K_m mitochondrial AlDH uses formaldehyde as a substrate whereas the high K_m enzyme does not. We, therefore, assayed AlDH activity in pargyline treated rats using formaldehyde and high and low propionaldehyde concentrations to investigate whether there is preferential inhibition of AlDH activity by this drug.

MATERIALS AND METHODS

Pargyline was a gift of Abbott Laboratories. All other chemicals were of the highest purity available commercially. Female Holtzman rats aged 69–95 days (180–275 g) were injected i.p. with normal saline or pargyline HCl in a vol. of 1 ml/kg body wt. Ninety min later ethanol, 2.5 g/kg, was injected i.p. as a 31.25% v/v solution in saline. At 30, 90 and 150 min

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Table 1. Mean blood ethanol levels after pargyline (mg% ± standard error)

Drug (mg/kg)	30 min	90 min	150 min
Control	319 ± 14 (7)	272 ± 10 (8)	222 ± 14 (8)
25	280 ± 34 (8)	258 ± 20 (7)	240 ± 19 (8)
50	332 ± 19 (8)	271 ± 15 (8)	246 ± 14 (8)
100	328 ± 31 (8)	274 ± 12 (8)	271 ± 9 (8)

after ethanol administration 40 μl of blood was collected from the retro-orbital sinus with a capillary pipet.

The blood samples were added to 0.96 ml of 0.01 mg% isopropanol (internal standard) in 16 × 100 mm test tubes with rubber stoppers. Internal standard solutions contained 25 mM thiourea to avoid possible spontaneous acetaldehyde formation in hemolysates. Tubes were kept on ice until they were incubated for 15 min at 65°. An Aliquot (0.55 ml) of head space gas was injected into a Beckman GC-45 gas chromatograph equipped with a Poropack Q column and a flame ionization detector. The flow rate of the carrier gas, helium, was 55 ml/min. The flow rates of hydrogen and air were, respectively, 44 and 300 ml/min. The column temperature was 137°, detector 173° and inlet 150°. Peak areas were computed with a Hewlett-Packard 3373B integrator and compared with standards which were prepared and run daily. There was a linear relation between peak areas and the amount of ethanol and acetaldehyde in standards. Acetaldehyde levels in standards were determined spectrophotometrically using yeast alcohol dehydrogenase (ADH, EC 1.1.1.1, Alcohol: NAD oxidoreductase) in 50 mM sodium phosphate pH 7.4 with 0.35 mM NAD.

Immediately after the last blood samples were taken, rats were decapitated and their livers were removed and rinsed with cold 0.25 M sucrose containing 2 mM mercaptoethanol and 10 mM sodium phosphate pH 7.4 (sucrose solution). Homogenates (15% w/v) were prepared in the sucrose solution using a motor-driven teflon pestle. After centrifugation at 750 *g* for 5 min, the supernatant was centrifuged for 10 min at 10,000 *g*. This supernatant was used for supernatant AlDH assays. A portion of this supernatant was centrifuged for 1 hr at 40,000 *g* and the resulting supernatant was used for ADH assays.

The pellet from the 10,000 *g* spin was washed twice with 15 ml of the sucrose solution and centrifuged 10 min at 10,000 *g*. This washed pellet was resuspended with a ground glass homogenizer in 1 mM sodium phosphate pH 7.4 containing 2 mM mercaptoethanol and 0.2% sodium deoxycholate in a vol. of 1 ml per g of wet wt liver. After centrifugation at 40,000 *g* for 1 hr the supernatant was used for mitochondrial AlDH assays.

Enzyme activities were determined by absorption changes at 340 nm with a Gilford 2400 spectrophotometer. AlDH reaction mixtures contained 50 mM sodium pyrophosphate pH 8.8, 1 mM pyrazole, 1 mM NAD and 0.05 ml of supernatant or mitochondrial fraction in a total vol. of 1 ml. Reactions were started by the addition of substrate. Blanks without aldehyde were run with all reactions. ADH assays were run in glycine-NaOH buffer pH 9.6 containing semicarbazide with 0.943 mM NAD and 0.033 ml supernatant. Reactions were started by adding 15.6 mM ethanol. Blanks contained 0.33 mM pyrazole and no ethanol.

Possible *in vitro* inhibition of AlDH was measured in three systems. Whole mitochondria, isolated as described earlier, 20% liver homogenates and liver slices were incubated in sucrose solution (0.25 M sucrose with 2 mM mercaptoethanol and 10 mM sodium phosphate, pH 7.4). Samples from the same animal were incubated with and without 1 mM pargyline. Preparations were incubated for 15 and 30 min at 0°, 25° and 37°. Tissues were then prepared as earlier described—homogenized, centrifuged and mitochondria disrupted with sodium deoxycholate. AlDH activities were monitored spectrophotometrically as previously described.

Samples, 5 ml, of supernatant after deoxycholate treatment were dialyzed 12 hr against 2 liters of 1 mM sodium phosphate pH 7.4 containing 2 mM mercaptoethanol. Both dialyzed and undialyzed samples were assayed for AlDH.

Proteins were determined by the biuret method [9]. Statistical comparisons were made with a one way analysis of variance using Duncan's mean range test ($P < 0.05$).

RESULTS

As illustrated by Table 1, control ethanol elimination rates are essentially linear. Rates increasingly deviate from linearity with increasing pargyline dose. However, pargyline-treated animals' ethanol levels are not significantly different from controls'. The large

Table 2. Mean blood acetaldehyde levels after pargyline treatment (μg/ml ± standard error)

Drug (mg/kg)	30 min	90 min	150 min
Control	3.32 ± 0.72 (7)	3.48 ± 1.12 (8)	2.57 ± 0.88 (7)
25	6.54 ± 1.17 (8)	6.32 ± 1.74 (8)	5.89 ± 2.08 (8)
50	8.90 ± 1.22 (8)*	8.69 ± 2.10 (8)	8.99 ± 2.29 (8)*
100	13.1 ± 1.70 (8)*,†,‡	9.39 ± 1.51 (8)	9.06 ± 1.60 (8)*

Values significantly different from:
* Control; † 25 mg/kg; ‡ 50 mg/kg.

Table 3. Supernatant enzyme activities after pargyline treatment (nmoles NADH produced/min/mg protein ± standard error)

Drug (mg/kg)	AlDH	ADH
Control	8.17 ± 0.68 (8)	7.71 ± 0.48 (8)
25	8.66 ± 1.07 (8)	8.01 ± 0.63 (8)
50	8.50 ± 0.78 (8)	8.75 ± 0.51 (8)
100	8.79 ± 0.61 (7)	8.29 ± 0.25 (7)

Table 4. Mitochondrial AlDH activity measured with 5 mM propionaldehyde after pargyline (nmoles NADH produced/min/mg protein ± standard error)

Drug (mg/kg)	Activity	% Inhibition
Control	28.9 ± 1.22 (8)	
25	23.6 ± 1.20 (8)*	18
50	19.7 ± 1.31 (8)	32
100	16.4 ± 1.04 (7)*,†	43

Values significantly different from:
* Control; † 25 mg/kg.

variation in ethanol levels within each group may mask any significant differences. There is a dose-dependent increase in blood acetaldehyde levels at all three time points (Table 2). Levels were significantly increased over control levels by 50 and 100 mg/kg pargyline at 30 and 150 min after ethanol administration. Treatment with 100 mg/kg pargyline resulted in acetaldehyde levels that were significantly higher than those produced by 50 mg/kg at 30 min after alcohol injection.

Supernatant AlDH and ADH activities were not significantly altered by pargyline treatment (Table 3). Mitochondrial AlDH activities were measured in animals sacrificed ½, 1, 2, 3, 4, 5, 6, 8 or 12 hr after pargyline injection. The same maximal inhibition was seen at 3, 4 and 5 hr time points. For this reason further experiments were run during this time period.

Total mitochondrial AlDH activities are shown in Table 4. Activity, measured with 5 mM propionaldehyde, was somewhat inhibited by pargyline, i.e. 43 per cent inhibition with 100 mg/kg treatment. This degree of inhibition might be expected if only the low K_m AlDH was inhibited. It has been reported that about one-half of the mitochondrial AlDH activity is due to activity of the low K_m enzyme [10–12]. At the highest pargyline dose AlDH activity measured with a low, 50 μM, propionaldehyde concentration inhibition was 84 per cent (Table 5). Even at this low aldehyde concentration the high K_m enzyme may contribute somewhat to aldehyde oxidation. At least three studies have indicated that formaldehyde in mitochondria is oxidized almost exclusively by the low K_m enzyme located inside the mitochondrial matrix [7, 8, 13]. We saw almost total (95 per cent) inhibition of activity with 1 mM formaldehyde in animals given 100 mg/kg pargyline.

No *in vitro* inhibition was seen with 1 mM pargyline as compared to controls incubated the same period of time at the same temperature. Pargyline did not affect AlDH activity of whole mitochondria, liver homogenates or liver slices incubated for 15 or 30 min at 0°, 25° or 37°.

Total proteins in dialyzed samples decreased by about one-third. For this reason AlDH specific activities in dialyzed samples increased about 50 per cent over their corresponding undialyzed controls. AlDH activity in dialyzed samples from animals given 100 mg/kg pargyline as compared with dialyzed controls was inhibited 45 per cent as measured with 5 mM propionaldehyde, 80 per cent with 50 μM propionaldehyde and 95 per cent with 1 mM formaldehyde. Since this is approximately the same percent inhibition seen in undialyzed samples relative to undialyzed controls (Tables 4 and 5) it can be concluded that dialysis did not reverse *in vivo* pargyline inhibition of AlDH activity.

DISCUSSION

The dose-dependent inhibition of mitochondrial low K_m AlDH activity and increase in blood acetaldehyde levels support previous investigations which sug-

Table 5. Low K_m mitochondrial AlDH activity after pargyline treatment (nmoles NADH produced/min/mg protein ± standard error)

Drug (mg/kg)	50 μM Propionaldehyde	Inhibition	1 mM Formaldehyde	Inhibition
Control	9.86 ± 0.62 (8)		14.9 ± 1.46 (8)	
25	5.78 ± 0.40 (8)*	52%	6.81 ± 0.71 (8)*	54%
50	3.09 ± 0.36 (8)*,†	69%	3.18 ± 0.55 (8)*,†	79%
100	1.57 ± 0.20 (7)*,†,‡	84%	0.814 ± 0.145 (7)*,†,‡	95%

Values significantly different from:
* Control; † 25 mg/kg; ‡ 50 mg/kg.

gest acetaldehyde is metabolized primarily by this enzyme. Dembiec *et al.* [2] found decreases in ethanol elimination after pargyline administration in mice. They used a higher alcohol dose, 4 g/kg, and their control blood ethanol levels were much higher than those seen in this study. Since it has been reported that approximately 20 per cent of total hepatic AlDH activity resides in the mitochondria of mice [14] and 45–80 per cent of AlDH activity in rat liver is located in mitochondria [5, 8], differences in ethanol blood levels and inhibition of AlDH activity in these two studies may also be due to differences in subcellular AlDH distribution in the two species.

Because blood ethanol levels were not significantly increased after pargyline treatment in our study, and in an earlier study ethanol levels did not increase to nearly the degree that sleep time did [1], pargyline may affect sleep time by some mechanism other than simply increasing blood ethanol levels. This is consistent with evidence from an earlier study in which pargyline-treated mice regained their righting reflex at blood levels significantly lower than those of controls [1].

It is possible that sleep time increases are due to well-characterized monoamine oxidase inhibition by pargyline. Neuroamine levels and turnover rates are most certainly affected by this drug. AlDH inhibition could also result in higher levels of potentially active aldehydes or their metabolites.

Increased acetaldehyde levels may also affect hypnosis [15]. Increased brain acetaldehyde levels would increase chances of forming alkaloid condensation products of acetaldehyde with biogenic amines. Collins and Bigdeli [16] reported *in vivo* production of salsolinol, the product of acetaldehyde and dopamine condensation, in the brains of ethanol-treated rats given pyrogallol to inhibit AlDH. It is interesting that administration of pargyline resulted in 7-fold higher concentrations of salsolinol in these animals.

Since dialysis did not abolish enzyme inhibition it might be suggested that AlDH inhibition by pargyline may not be reversible. Our continuing *in vitro* pargyline studies may provide additional information on its mechanism of inhibition.

The differential enzyme inhibition reported in this paper helps to explain previously observed effects of pargyline on acetaldehyde accumulation [2, 3]. It also explains failure to observe AlDH inhibition comparable to acetaldehyde increases when enzyme activity was measured using millimolar concentrations of propionaldehyde or acetaldehyde as substrate [2].

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