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EFFECTS OF ALDEHYDE DEHYDROGENASE INHIBITORS ON ENZYMES INVOLVED IN THE METABOLISM OF BIOGENIC ALDEHYDES IN RAT LIVER AND BRAIN

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Abstract—The effects of the aldehyde dehydrogenase inhibitors disulfiram, coprine and cyanamide on enzymes involved in the metabolism of biogenic aldehydes in rat liver and brain were studied. Both liver and brain aldehyde dehydrogenase activities were significantly decreased in rats pretreated with these drugs. In the liver, the low-K_m aldehyde dehydrogenase activity was markedly decreased by all three drugs after 2 and 24 hr whereas only cyanamide inhibited the high-K_m enzymes. The brain ALDH-activity with a low acetaldehyde concentration was significantly decreased by coprine and cyanamide at both times tested, whereas disulfiram caused no change after 2 hr but an inhibition of 38% after 24 hr. The brain ALDH-activity with a high acetaldehyde concentration was significantly decreased by coprine and cyanamide but not by disulfiram. The activity of the substrate specific enzyme succinate semialdehyde dehydrogenase in brain was slightly but significantly decreased in rats pretreated with cyanamide but not in rats pretreated with disulfiram or coprine. None of the drugs caused any changes in the activities of aldehyde reductase and monoamine oxidase in brains *in vivo*. The activity of monoamine oxidase in liver was significantly decreased by coprine after 24 hr. In contrast to the effects obtained *in vivo*, disulfiram was found to be an inhibitor *in vitro* of brain succinate semialdehyde dehydrogenase and liver monoamine oxidase. Aldehyde reductase was slightly inhibited by both disulfiram and 1-aminocyclopropanol *in vitro*.

Various compounds are known to cause hypersensitivity to ethanol. Disulfiram (Antabuse®) and cyanamide, in the form of citrated calcium carbimide (Dipsan®, Temposil®), have been used in alcohol therapy for many years. This therapy is based on the alcoholic's fear of the unpleasant physiological reactions elicited after ethanol ingestion (for reviews see refs. [1–3]). A similar reaction to ethanol has also been observed in humans who have eaten the mushroom *Coprinus atramentarius*, and in experimental animals pretreated with coprine (N^5-(1-hydroxycyclopropyl)-L-glutamine), the active compound isolated from this mushroom [4–6]. These three drugs are inhibitors of liver aldehyde dehydrogenase (ALDH) *in vivo* and cause an accumulation of acetaldehyde in the body during ethanol oxidation. Although it has been clearly established that the disulfiram–ethanol reaction (DER) is associated with an increased aldehyde level, the mechanism of the DER is not yet fully understood [1, 6, 7].

The role of ALDH in the DER has not been discussed as much as the role of acetaldehyde. Apart from being responsible for the oxidation of acetaldehyde in the liver, this enzyme is also responsible for the oxidation of biogenic aldehydes in CNS and peripheral tissues. These aldehydes are formed from the oxidative deamination of biogenic amines, catalysed by monoamine oxidase (MAO). In the CNS of man and rat, the aldehyde from noradrenaline is primarily reduced to an alcohol metabolite by a NADPH-dependent aldehyde reductase, whereas the aldehydes from dopamine and serotonin are primarily oxidized to acid metabolites by a NAD-dependent ALDH [8, 9].

Several studies have shown that disulfiram as well as cyanamide inhibit ALDH in rat brain *in vivo* [10–15]. It has also been reported that disulfiram interferes with the metabolism of serotonin *in vivo*, causing a reduction of serotonin turnover and an impairment of the elimination of its metabolites [16, 17]. Recently it was demonstrated that rat liver MAO was inhibited by disulfiram *in vitro* [18]. This inhibition was potentiated by ethanol and it was suggested that this synergistic effect of disulfiram and ethanol on MAO could be responsible for the DER. Thus, a change in the overall metabolism of biogenic amines caused by disulfiram may be an important factor involved in the DER. The present study was undertaken to further investigate the effects, *in vivo* and *in vitro*, of disulfiram and two other alcohol-sensitizing compounds, cyanamide and coprine, on enzymes involved in the metabolism of biogenic aldehydes.

MATERIALS AND METHODS

Chemicals. Disulfiram and acetaldehyde were supplied by Fluka, Buchs, Switzerland. Disulfiram was recrystallized twice in 99.5% ethanol. Acetaldehyde was freshly distilled before use. Cyanamide was obtained from Schuchardt, Munich, West Germany. Coprine (N^5-(1-hydroxycyclopropyl)-L-glutamine) and 1-aminocyclopropanol, synthesized according to Lindberg *et al.* [19], were a gift from Astra Pharmaceuticals. Succinate semialdehyde was obtained from Sigma Biochemicals, St. Louis, U.S.A. All other chemicals were obtained from Sigma Biochemicals, and E. Merck, Darmstadt, West Germany.

Animals. Female Sprague–Dawley rats (Anticimex, Sollentuna, Sweden) weighing 200–250 g, were

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used in all experiments. The rats had free access to food and tap water.

Drug administration. All drugs were administered by intraperitoneal injections, 2 and 24 hr before the measurements. Disulfiram was suspended in 5% (w/w) gum arabicum (*Acacia*) and given in a dose of 150 mg/kg. Coprine and cyanamide were dissolved in saline and given in doses of 30 mg/kg. Control rats received the corresponding volumes of saline or gum arabicum.

Tissue preparation and enzyme assays. The rats were killed and livers and brains were quickly removed. The livers were homogenized in a medium (pH 7.2) containing 0.25 M sucrose, 0.5 mM EDTA and 5 mM Tris–HCl. The brains were homogenized in ice-cold saline (0.9%).

The aldehyde dehydrogenase (ALDH) activity in liver was assayed spectrophotometrically by measuring the reduction of NAD^+ at 340 nm [20]. The activity of the low-K_m enzyme and the total activity of ALDH were assayed with 25 μM and 5 mM of acetaldehyde, respectively. The difference between the total activity and the activity of the low-K_m enzyme represents the activity of the high-K_m enzymes [21, 22].

Succinate semialdehyde dehydrogenase activity in brain was determined by the same method with succinate semialdehyde (35 μM) as the substrate. The effects *in vitro* of the inhibitors on this enzyme were measured with the same method in experiments on brain mitochondria isolated according to Pettersson and Tottmar [23]. Mitochondria were incubated with 2–40 μM disulfiram (dissolved in methanol) or 1 mM cyanamide, coprine and 1-aminocyclopropanol, respectively, for 10 min at room temperature before the reaction was started with the substrate.

The ALDH-activity in brain homogenates with a low concentration of acetaldehyde (20 μM) was determined as the rate of disappearance of acetaldehyde as previously described [14]. The recovery of acetaldehyde from tissue- and water-blanks was close to 100% showing that no or very little acetaldehyde was lost through tissue binding or evaporation. The total activity of brain ALDH was measured by the same method as for the liver with 5 mM acetaldehyde. Samples of liver and brain homogenates were solubilized with sodium deoxycholate (0.5%, w/v, final concentration) shortly before the assay.

Aldehyde reductase activity was determined by following the oxidation of NADPH spectrophotometrically at 340 nm with 0.5 mM *p*-nitrobenzaldehyde as substrate [24]. The effects of the inhibitors of this enzyme *in vitro* were measured fluorimetrically in experiments on a brain cytosolic fraction isolated according to Pettersson and Tottmar [23]. The reaction mixture contained 0.1 M phosphate buffer (pH 7.0), enzyme solution, 10 μM NADPH and 40 μM disulfiram (dissolved in methanol) or 1 mM cyanamide, coprine and 1-aminocylocopropanol, respectively. After incubation for 10 min at 25° the reaction was started by the addition of 80 μM *p*-nitrobenzaldehyde.

Monoamine oxidase (MAO) activity was measured fluorimetrically by following the formation of 4-hydroxyquinoline from kynuramine at 37° in 0.3 M K^+-phosphate buffer (pH 7.4) [25]. MAO-activity in liver was also determined with benzylamine as substrate (2 mM) by following the formation of benzaldehyde spectrophotometrically at 250 nm in 50 mM phosphate buffer (pH 7.5) according to Tabor *et al.* [26]. The activity of MAO in the *in vitro* experiments on liver mitochondria was measured by the last method. The mitochondrial fraction was isolated from a 10% (w/v) liver homogenate in 0.25 M sucrose medium [21]. Samples of mitochondria were solubilized with sodium deoxycholate (0.5%, final concentration) shortly before the assay. The mitochondrial samples were preincubated at room temperature for 1–30 min in the presence of 5–50 μM disulfiram (dissolved in dimethylsulfoxide, DMSO) before the addition of benzylamine. In control tests, DMSO and ethanol were added to the same final concentration.

Protein was determined with bovine serum albumin as standard [27]. Statistical analysis were performed by the Wilcoxon test for two independent samples [28]. The effect of ethanol on MAO-activity was analysed by linear regression. The estimated slope of the regression line was tested by an analysis of variance with a *t*-test [28].

RESULTS

Effects on ALDH, succinate semialdehyde dehydrogenase and aldehyde reductase

In vivo. Disulfiram, cyanamide and coprine were administered in doses which according to previous studies [5, 29] should inhibit the liver low-K_m ALDH by 50–90% after a pretreatment time of 2–24 hr. Disulfiram caused a greater inhibition after 24 hr than after 2 hr, whereas the opposite effect was obtained with cyanamide. The inhibition with coprine was slightly greater after 24 hr than after 2 hr. The high-K_m activity of ALDH in the liver was markedly decreased by cyanamide but unaffected by disulfiram and coprine.

The ALDH-activity in brain with the low acetaldehyde concentration (20 μM) was also decreased by the drugs. The inhibition pattern was rather similar to that found in the liver (Table 1). However, the inhibition was less pronounced, and disulfiram caused no inhibition after 2 hr. The ALDH-activity in brain with the high acetaldehyde concentration (5 mM) was decreased by cyanamide and coprine but not by disulfiram.

No decrease in the ALDH-activity was found when liver or brain homogenates from control rats were preincubated at room temperature with homogenates from drug-treated rats. This showed that the homogenates contained no, or very low, levels of the drugs in their free form.

The activity of succinate semialdehyde dehydrogenase in brain was unchanged in rats pretreated with disulfiram and coprine (Table 2). A slight, but significant inhibition of this enzyme was observed after pretreatment with cyanamide. The activity of the NADPH-dependent aldehyde reductase in brain was not affected by any of these drugs (Table 2).

In vitro. Previous studies in our laboratory ([29] and unpublished results) have shown that the low-

Table 1. Effects of disulfiram, coprine and cyanamide on brain and liver aldehyde dehydrogenase activities *in vivo*†

	Drug treatment	Low substrate concentration				High substrate concentration (total activity)			
		2 hr	% inhibition	24 hr	% inhibition	2 hr	% inhibition	24 hr	% inhibition
Brain	Control	0.23 ± 0.02	—	0.24 ± 0.05	—	2.41 ± 0.29	—	2.07 ± 0.24	—
	Disulfiram	0.20 ± 0.03	13	0.15 ± 0.04*	37	2.13 ± 0.20	12	2.02 ± 0.17	2
	Coprine	0.07 ± 0.01**	70	0.08 ± 0.02**	66	1.69 ± 0.13**	30	1.57 ± 0.26*	24
	Cyanamide	0.07 ± 0.01**	70	0.16 ± 0.01**	33	1.44 ± 0.05*	40	1.43 ± 0.14**	31
		Low substrate concentration (low K_m-ALDH)				High substrate concentration (high K_m-ALDH)			
Liver	Control	6.1 ± 0.4	—	5.8 ± 0.1	—	12.4 ± 1.0	—	11.8 ± 1.1	—
	Disulfiram	3.5 ± 0.3**	43	1.6 ± 0.3**	72	13.0 ± 0.1	—	12.5 ± 0.3	—
	Coprine	1.0 ± 0.1**	84	1.6 ± 0.1**	72	12.1 ± 0.5	—	12.9 ± 1.5	—
	Cyanamide	0.2 ± 0.2**	97	3.3 ± 0.4**	43	2.7 ± 0.3**	78	7.2 ± 0.6**	39

* Significantly different from control values, $P \leq 0.05$.

** Significantly different from control values, $P \leq 0.01$.

† Rats were given disulfiram (150 mg/kg), coprine (30 mg/kg) or cyanamide (30 mg/kg) 2 or 24 hr before measurements. The activities of brain ALDH are expressed as nmoles of NADH/min/mg protein (high substrate concentration) and nmoles of acetaldehyde oxidized/min/mg protein (low substrate concentration). The activities of liver ALDH are given as nmoles NADH/min/mg protein. Values are the means ± S.D. of 3–5 experiments.

Table 2. Effects of disulfiram, coprine and cyanamide on succinate semialdehyde dehydrogenase and aldehyde reductase in brain *in vivo**

	Succinate semialdehyde dehydrogenase (nmoles NADH/min/mg protein)		Aldehyde reductase (nmoles NADPH/min/mg protein)	
Drug	2 hr	24 hr	2 hr	24 hr
Control	11.4 ± 0.6	11.2 ± 0.6	2.46 ± 0.22	2.31 ± 0.13
Disulfiram	11.2 ± 0.9	11.5 ± 1.3	2.40 ± 0.18	2.33 ± 0.14
Coprine	10.7 ± 1.2	10.8 ± 1.2	2.28 ± 0.39	2.38 ± 0.19
Cyanamide	9.1 ± 0.9†	9.9 ± 0.3†	2.27 ± 0.17	2.32 ± 0.12

* The enzyme activities in brain homogenates were assayed 2 and 24 hr after administration of disulfiram (150 mg/kg), coprine (30 mg/kg) or cyanamide (30 mg/kg). The values are the means ± S.D. from five experiments.

† Significantly different from control values ($P < 0.01$).

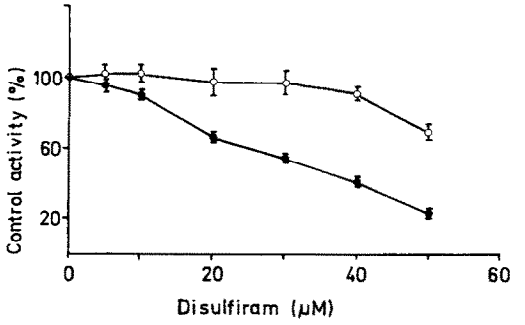

Fig. 1. Dose–response curve for inhibition of rat liver monoamine oxidase by disulfiram *in vitro*. The reaction mixture contained 50 mM sodium pyrophosphate buffer, pH 8.8 (solid circles) or 50 mM phosphate buffer, pH 7.5 (open circles), enzyme solution and disulfiram (5–50 μM in DMSO). After incubation at room temperature for 20 min, the reaction was initiated by the addition of 2 mM benzylamine. Control activities were 4.9 ± 0.1 and 7.3 ± 0.1 nmoles benzaldehyde formed/min/mg protein at pH 7.5 and 8.8, respectively. Values are the means ± S.D. from three determinations.

K_m ALDH present in rat liver and brain is irreversibly inhibited *in vitro* by disulfiram, cyanamide and 1-aminocyclopropanol (the active metabolite of coprine). No further experiment *in vitro* with ALDH was performed in the present study.

In contrast to the results obtained *in vivo*, disulfiram was found to be a potent inhibitor of brain succinate semialdehyde dehydrogenase *in vitro*.

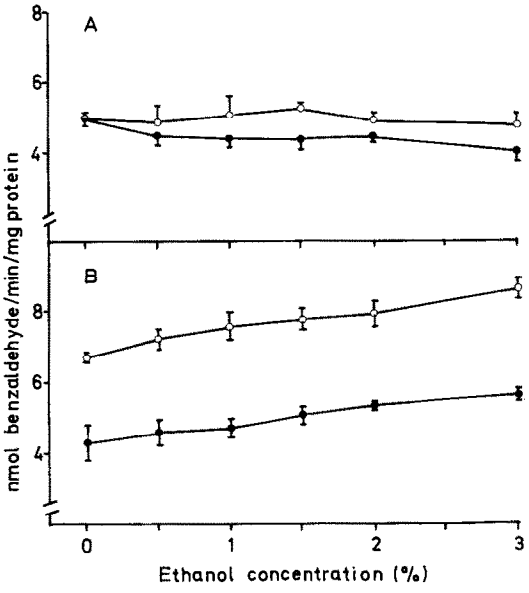

Fig. 2. The effect of ethanol on the inactivation of rat liver monoamine oxidase by disulfiram *in vitro*. The mitochondria were incubated with disulfiram (30 μM in DMSO) and ethanol at various concentrations for 20 min at room temperature before the reaction was initiated by the addition of 2 mM benzylamine (solid circles). In the control tests with benzylamine, ethanol and DMSO were included at the same final concentration (open circles). Values represent means ± S.D. from at least three experiments. (A) pH 7.5; (B) pH 8.8.

Addition of disulfiram to a preparation of brain mitochondria caused a rapid decline in the enzyme activity during the first 2 min, followed by a slow, progressive decline. Almost total inhibition was obtained after 10 min with 10 μM disulfiram, and a 50% inhibition was obtained with 5 μM disulfiram. The degree of inhibition observed after 10 min with 5 μM disulfiram was similar after a 10-fold dilution of the enzyme–inhibitor mixture, which showed that disulfiram was firmly bound to the enzyme. Addition of succinate semialdehyde to the assay mixture before disulfiram did not protect the enzyme from inhibition.

Cyanamide and 1-aminocyclopropanol were much less effective than disulfiram as inhibitors of succinate semialdehyde dehydrogenase. The inhibition was 20–30% after 10 min preincubation with these drugs at a concentration of 1 mM. Coprine caused no inhibition *in vitro*.

The effects of the drugs on brain aldehyde reductase *in vitro* was studied fluorimetrically in experiments on a cytosolic fraction isolated from the brain homogenate. The activity was measured at non-saturating concentrations of NADPH and *p*-nitrobenzaldehyde in order to reduce a large NADPH-dependent blank reaction and a quenching of the fluorescence caused by *p*-nitrobenzaldehyde. The activity of aldehyde reductase was slightly decreased (15–25%) after a preincubation time of 10 min in the presence of 40 μM disulfiram and 1 mM 1-aminocyclopropanol. Cyanamide and coprine did not affect the enzyme activity.

Effects on MAO-activity in vivo

The activity of MAO in liver was determined with two different substrates. Disulfiram and cyanamide caused no changes in the MAO-activity in rats pretreated for 2 or 24 hr. Pretreatment with coprine for 24 hr significantly decreased the activity by 32% (0.94 ± 0.20, $n = 8$, and 0.64 ± 0.05, $n = 6$, nmoles/min/mg protein, respectively, $P < 0.01$) when benzylamine was used as substrate, but not with kynuramine as the substrate. None of the three drugs tested had any significant effects on the MAO-activity in brain after 2 or 24 hr.

Effects of disulfiram and ethanol on MAO-activity in vitro

The effects of disulfiram and ethanol on the activity of the mitochondrial MAO in liver were studied at two different pH-values. The control activity of MAO was approx. 1.5-times higher at pH 8.8 than at pH 7.5. Disulfiram inhibited the enzyme more markedly at the higher pH-value (Fig. 1). The inhibition was dose-dependent and increased with the time of preincubation (not shown). Addition of ethanol, up to a concentration of 3% (v/v), increased the control activity at pH 8.8 in a dose-dependent way (Fig. 2b). Ethanol also stimulated the MAO-activity in the presence of disulfiram. Linear regression analysis showed that MAO-activity both in the presence and absence of disulfiram correlated significantly with the ethanol concentration ($P < 0.001$), and that the estimated slope of the regression line was significantly different from zero ($P < 0.01$). Thus, the inhibition by disulfiram remained rather

constant (33–38%) at the different concentrations of ethanol. At pH 7.5, the enzyme activity was unaffected by ethanol both in the presence and absence of disulfiram (Fig. 2a).

In some separate experiments the effects of coprine and aminocyclopropanol on MAO-activity were tested in a similar way. Neither of them, at concentrations of 0.1 mM, caused any changes in the activity at pH 7.5 or 8.8.

DISCUSSION

In agreement with previous results it was found in the present study that all three drugs caused a pronounced inhibition of the low-K_m ALDH in liver *in vitro* but that only cyanamide inhibited the high-K_m ALDH [29].

Low- and high-K_m forms of ALDH are also present in rat brain [23]. The ALDH-activity in brain at the low acetaldehyde concentration, which probably mainly represents the activity of the low-K_m ALDH [23], was less sensitive to drugs than the liver enzyme. In contrast to this, Berger and Weiner [11] reported that disulfiram caused a greater inhibition of ALDH in the brain than in the liver of rats. They measured the activity indirectly by following the formation of 3,4-dihydroxyphenylacetic acid from dopamine in tissue slices. However, in experiments with brain- and liver-mitochondria from disulfiram-treated rats, no significant difference in inhibition was found when the activity was assayed directly with a low concentration (5 μM) of 3,4-dihydroxyphenylacetaldehyde (Pettersson, H. and Tottmar, O., unpublished results). In the study of Berger and Weiner [11], disulfiram caused an increased deamination rate of dopamine in the liver slices but not in the brain slices. Thus, the concentration of the aldehyde in the liver slices from the disulfiram-treated rats might have increased to a level where the high-K_m forms of ALDH are operative. Since the high-K_m activity is largely unaffected by disulfiram, the rate of oxidation of the aldehyde would appear to be less affected by disulfiram in this situation, thereby suggesting a smaller inhibition of ALDH.

In a study on cyanamide-treated mice, Deitrich *et al.* [10] found a more marked difference in sensitivity of liver and brain ALDH than that observed in the present study on rats given a similar treatment with cyanamide. They measured the total ALDH-activity at a high concentration of acetaldehyde, and therefore, no conclusion can be made about the relative sensitivity of the low- and high-K_m ALDH present in the brain and liver of mice. It appears from the results shown in Table 1 that the difference in cyanamide sensitivity between liver and brain ALDH will be more marked when the activity is measured at a high acetaldehyde concentration than at a low concentration.

A different degree of inhibition of brain ALDH at the low and high substrate concentrations was also observed by Minegishi *et al.* [13] in rats pretreated with disulfiram. The inhibition of the low-K_m enzyme by disulfiram is rather difficult to detect if the activity is measured at a high substrate level, especially in brain, since the low-K_m enzyme constitutes only 10–15% of the total ALDH-activity ([23], see also Table 1).

Succinate semialdehyde dehydrogenase is a specific form of ALDH involved in the catabolism of γ-aminobutyric acid [30, 31]. Succinate semialdehyde dehydrogenase occurs in great excess in rat brain [32], and it appears unlikely that the slight inhibition in cyanamide-treated rats would affect the level of γ-aminobutyric acid.

The inhibition of succinate semialdehyde dehydrogenase *in vitro* by disulfiram appeared to be irreversible, since the reaction between the enzyme and disulfiram was clearly time-dependent, and dilution of the enzyme–inhibitor complex did not affect the degree of inhibition. This seems to exclude the possibility that the unchanged activity found in brain homogenates was merely due to a dissociation of the enzyme–inhibitor complex on dilution.

None of the drugs tested had any effect *in vivo* on the activity of aldehyde reductase in brain. The results obtained with disulfiram are in agreement with previous studies performed *in vivo* on rats [13] and *in vitro* with aldehyde reductase from human brain [33]. In the study by Minegishi *et al.* [13] and in the present study, the activity of aldehyde reductase was determined with a relative high concentration of *p*-nitrobenzaldehyde. Since both high- and low-K_m forms of aldehyde reductase have been reported to be present in rat brain [24], some effects by these drugs on the low-K_m enzyme cannot be excluded.

It was recently shown that MAO of rat liver mitochondria was inhibited by disulfiram *in vitro* [18], and a similar effect of disulfiram was observed in our study. The inhibition was most pronounced at a high, unphysiological pH, and this may explain the lack of inhibition *in vivo*. Ethanol stimulated the MAO-activity *in vitro* both in the presence and absence of disulfiram at pH 8.8 but not at pH 7.5. In sharp contrast to this, Schurr *et al.* [18] found that ethanol potentiated the inhibition by disulfiram at pH 7.4 but decreased the inhibitory effect of disulfiram at pH 9.1. They suggested that this inhibition of MAO, potentiated by ethanol, as well as the known inhibition of dopamine-β-hydroxylase by disulfiram may lead to increased levels of dopamine, which together with acetaldehyde may be responsible for the DER. In view of the results obtained in the present study, and in previous studies on disulfiram-treated rats [17, 34], it seems unlikely, however, that inhibition of MAO by disulfiram would be an important factor in the DER.

Acetaldehyde is a poor substrate or inhibitor for aldehyde reductase, at least in pig and bovine brain [35–37]. On the other hand, *in vitro* studies have shown that acetaldehyde at low concentrations (K_i = 2.6 μM) competitively inhibits the metabolism of 5-hydroxyindoleacetaldehyde (the aldehyde from serotonin) by rat brain ALDH [38]. Since the K_m for acetaldehyde is in the order of 1 μM [23, 31, 39], very low concentrations of acetaldehyde could inhibit the metabolism of biogenic aldehydes. It has been suggested that brain ALDH may function as a metabolic barrier to prevent an accumulation of acetaldehyde [40, 41]. Thus, during normal metabolism of ethanol, ALDH in brain could maintain the con-

centration of acetaldehyde at an extremely low level. It has been shown, however, that after ethanol administration to rats pretreated with disulfiram, the levels of acetaldehyde were increased in blood as well as in brain, whereas no acetaldehyde was detected in brain of rats receiving ethanol only [42]. If the observed competitive inhibition of ALDH by acetaldehyde also occurs *in vivo*, this means that brain ALDH can be inhibited by both disulfiram and acetaldehyde during the DER. The oxidative pathway might then become rate limiting and lead to increased steady-state levels of biogenic aldehydes or to an increased reduction of the aldehydes to the corresponding alcohols catalysed by aldehyde reductase [43, 44]. Several studies have shown that biogenic aldehydes themselves have pharmacological properties [45–48] and are particularly important in the regulation of sleep [49, 50].

Another possibility is that accumulation of biogenic aldehydes might result in a condensation between biogenic amines and their aldehyde derivatives, or with acetaldehyde, to form alkaloid products (for a review see ref. [51]). These products have been shown to affect uptake, storage, release and metabolism of biogenic amines and to act as false neurotransmitters [51]. So far, the occurrence of condensation products in brain has mainly been demonstrated after drug administration leading to increased acetaldehyde levels. Since all three drugs tested in this study may promote an accumulation of both biogenic aldehydes and acetaldehyde due to inhibition of ALDH in brain and liver, it is pertinent to speculate on the possible role of these condensation products in the alcohol reaction.

In summary, these results demonstrate that ALDH in brain is inhibited in rats pretreated with disulfiram, cyanamide and coprine, whereas the other enzymes involved in the metabolism of biogenic aldehydes are more or less unaffected by these drugs. The inhibition may alter the metabolism of biogenic amines in several ways: (1) by increasing the levels of biogenic aldehydes; (2) by inducing a reductive shift towards alcohol metabolites; or (3) by promoting the formation of condensation products. Thus, disulfiram and other ALDH-inhibitors may cause a serious change in the overall metabolism of biogenic amines. What the long-term effects of such an alteration would be is difficult to predict, but it is appropriate to assume that some of the side-effects associated with disulfiram treatment are related to its effects on biogenic amine metabolism. Whether the effect on brain ALDH is involved in the mechanism underlying the DER is at the present time uncertain.

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