Human Class I Alcohol Dehydrogenases Catalyze the Interconversion of Alcohols and Aldehydes in the Metabolism of Dopamine†

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ABSTRACT: The class I human liver alcohol dehydrogenases (ADHs) catalyze the interconversion of the intermediary alcohols and aldehydes of dopamine metabolism in vitro, whereas those of the class II and class III do not. The individual, homogeneous class I isozymes oxidize (3,4-dihydroxyphenyl)ethanol and (4-hydroxy-3-methoxyphenyl)ethanol (HMPE) and ethanol with k_{cat}/K_m values in the range from 16 to 240 mM^{-1} min^{-1} and from 16 to 66 mM^{-1} min^{-1}, respectively. They reduce the corresponding dopamine aldehydes (3,4-dihydroxyphenyl)acetaldehyde and (4-hydroxy-3-methoxyphenyl)acetaldehyde (HMPAL) with k_{cat}/K_m values varying from 7800 to 190 000 mM^{-1} min^{-1}, considerably more efficient than the reduction of acetaldehyde with k_{cat}/K_m values from 780 to 4900 mM^{-1} min^{-1}. For $\beta_1\gamma_2$ ADH, ethanol competes with HMPE oxidation with a K_i of 23 μM. In addition, 1,10-phenanthroline inhibits HMPE oxidation and HMPAL reduction with K_i values of 20 μM and 12 μM, respectively, both quite similar to that for ethanol, K_i = 22 μM. Thus, both ethanol/acetaldehyde and the dopamine intermediates compete for the same site of ADH, a basis for the ethanol-induced in vivo alterations of dopamine metabolism.

Following the ingestion of ethanol, the concentrations of alcohol and acid metabolites of the biogenic amines dopamine, norepinephrine, and serotonin in tissues and body fluids change substantially. Attempts to explain the increased amounts of the alcohol metabolites have not been definitive. ADH[1] metabolizes the reduction of intermediary aldehydes formed by monoamine oxidase and subsequently oxidizes the resultant alcohols to acids, much as observed in norepinephrine metabolism (Mårdh et al., 1981). We have previously reported that class I ADH oxidizes norepinephrine glycols (Mårdh et al., 1985) and have now expanded these studies to dopamine. The results suggest that class I ADH interconverts dopamine alcohols and aldehydes.

Materials and Methods

Preparation of Aldehydes. The dopamine aldehydes (3,4-dihydroxyphenyl)acetaldehyde (DHPAL) and (4-hydroxy-3-methoxyphenyl)acetaldehyde (HMPAL) were synthesized with a pinacol–pinacolone rearrangement by acid treatment of the corresponding β-hydroxylated amines (norepinephrine and normetanephrine, respectively) (Fellman, 1958; Robbins, 1966). The purity of the aldehydes was verified by HPLC (Waters Inc.), with a methanol/10 mM phosphate buffer (pH 7.5) gradient from 10 to 80% methanol, on a Waters Radial-PAK NOVA-PAK C_{18} column. Both DHPAL and HMPAL eluted as single peaks. Final identification was performed with ammonia CI mass spectrometry on a Finnegan MAT 312 mass spectrometer, direct inlet, yielding ions of m/z 153, 170, and 187 for DHPAL and 167, 184, and 201 for HMPAL, which correspond to m/z $[M + H]^+$, $[M + NH_4]^+$, and $[M + NH_3 + NH_4]^+$, respectively.

ADH Isozymes. Human liver alcohol dehydrogenase isozymes were isolated, purified to homogeneity, and characterized as previously described (Wagner et al., 1983, 1984; Ditlow et al., 1984). The present study was performed with $\alpha\alpha$, $\alpha\beta_1$, $\beta_1\beta_1$, $\alpha\gamma_1$, $\alpha\gamma_2$, $\beta_1\gamma_1$, $\beta_1\gamma_2$, and $\gamma_2\gamma_2$ of class I ADH and class II (π) and class III (χ) ADH.

Measurement of Kinetic Constants. Changes in absorbance at 340 nm were monitored with a Gilford 2600 spectrophotometer. Assays were carried out in 0.1 M sodium phosphate, pH 7.4 at 25 °C, and with ADH-saturating concentrations of NAD^+ or NADH, 2.5 mM and 0.25 mM, respectively. Kinetic parameters were calculated from duplicate determinations of initial reaction rates from 6 to 10 different substrate concentrations, from 0.05 to 4.0 mM for DHPE and HMPE and from 0.25 to 5.0 mM for ethanol. The apparent K_m and k_{cat} values at these substrate concentrations were determined from Lineweaver–Burk plots. For kinetic determination, the synthetic aldehydes were used within 6 h of preparation. The aldehyde concentration in the assay solution was determined spectrophotometrically by allowing the reaction to go to completion.

Materials. Norepinephrine, normetanephrine, (4-hydroxy-3-methoxyphenyl)ethanol, NAD^+ (grade III), and NADH (grade III) from Sigma Chemical Co., St. Louis, MO, and (3,4-dihydroxyphenyl)ethanol from Chemalog, Chemical Dynamics Corp., NJ, were used as purchased.

Results

Class I isozymes of human liver ADH effectively catalyze both the oxidation of the dopamine intermediary alcohols, (3,4-dihydroxyphenyl)ethanol (DHPE) and (4-hydroxy-3-methoxyphenyl)ethanol (HMPE), and the corresponding reductions (Tables I and II), but class II or III ADH does not. Table I shows the kinetic parameters for the oxidation of DHPE and HMPE at pH 7.40, 25 °C, by the eight ADH isozymes examined at enzyme-saturating concentrations of NAD^+. The physiological pH chosen for these studies obviates

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[1] Abbreviations: ADH, alcohol dehydrogenase; DHPAL, (3,4-dihydroxyphenyl)acetaldehyde; DHPE, (3,4-dihydroxyphenyl)ethanol; HMPAL, (4-hydroxy-3-methoxyphenyl)acetaldehyde; HMPE, (4-hydroxy-3-methoxyphenyl)ethanol; OP, 1,10-phenanthroline.

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Table I: Kinetic Parameters for ADH Isozymes with Dopamine Alcohols and Ethanol[a]

		DHPE			HMPE			ethanol[b]		
class	isozyme	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m
I	$\alpha\gamma_1$	12	98	120	7.6	100	76	11	320	33
	$\alpha\gamma_2$	9	400	23	9.9	42	240	9.8	160	62
	$\alpha\beta_1$	2.8	15	190	2.9	180	16	39	980	40
	$\beta_1\gamma_1$	2.8	120	23	6.9	68	100	30	1100	26
	$\beta_1\gamma_2$	2.5	130	19	8.0	40	200	14	210	66
	$\alpha\alpha$		ND		2.3	220	10	11	400	28
	$\beta_1\beta_1$	0.7	140	5.0	0.2	230	0.87	9.1	550	16
	$\gamma_2\gamma_2$		ND		11	92	120		ND	
II	π		NA			NA		470[c]	120000[c]	3.9[c]
III	χ		NA			NA				0.002[c]

[a] NA, no activity noted at ≤8.0 mM substrate and 1.0 μM enzyme concentration; ND, not determined. Units are as follows: k_{cat}, min^{-1}; K_m, μM; k_{cat}/K_m, mM^{-1} min^{-1}. Values are at pH 7.4, 25 °C, at 0.05–4.0 mM substrate concentration. [b] Data from Mårdh et al. (1985) at 0.25–5.0 mM ethanol. [c] Data from Ditlow et al. (1984), pH 10.0.

Table II: Kinetic Parameters for Various ADH Isozymes with Dopamine Aldehydes and and Acetaldehyde[a]

		DHPAL			HMPAL			acetaldehyde[b]		
class	isozyme	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m
I	$\alpha\gamma_1$	1800	130	14000	2300	80	29000	1100	1300	850
	$\alpha\beta_1$	780	35	22000	390	50	7800	630	150	4200
	$\beta_1\gamma_1$	1700	8.9	190000	960	11	87000	860	1100	780
	$\beta_1\gamma_2$	4100	150	27000	2000	240	8300	1100	280	3900
	$\beta_1\beta_1$	28	720	39	4.4	35	130	380	76	4900
II	π		NA			NA		650	8300	80
III	χ		NA			NA			NA	

[a] NA, no activity noted at ≤1.0 mM substrate and 1.0 μM enzyme concentration. Units are as follows: k_{cat}, min^{-1}; K_m, μM; k_{cat}/K_m, mM^{-1} min^{-1}. Values are at pH 7.4, 25 °C, at 10–400 μM DHPAL and HMPAL. [b] Data from Deetz et al. (1984).

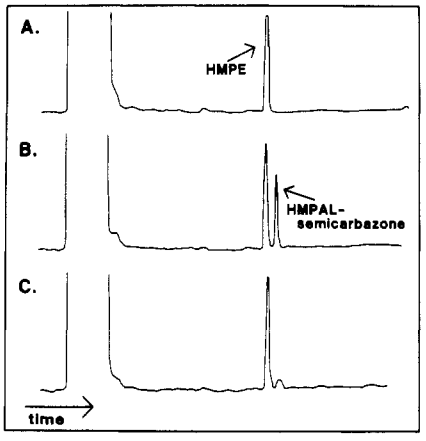

FIGURE 1: HPLC elution profiles of HMPE incubation mixtures after 15 min (A) without $\beta_1\gamma_2$ ADH, (B) with 1.5 μM $\beta_1\gamma_2$ ADH, and (C) with 1.5 μM $\beta_1\gamma_2$ ADH in the presence of 1.0 mM ethanol. Conditions: 100 μM HMPE; 2.5 mM NAD^+; 10 mM semicarbazide; 0.1 M phosphate; pH 7.40; 37 °C.

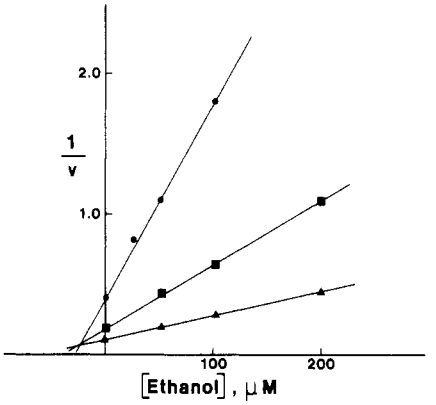

FIGURE 2: Inhibition of $\beta_1\gamma_2$ ADH oxidation of HMPE by ethanol. Reciprocal amounts, $[\mu mol\ h^{-1}\ (mg\ of\ protein)^{-1}]^{-1}$, of HMPAL semicarbazone produced within 15-min incubation are plotted vs. initial ethanol concentration in assays containing 25 (●), 50 (■), and 100 (▲) μM HMPE, 10 mM semicarbazide, 1.5 μM $\beta_1\gamma_2$ ADH, 0–200 μM ethanol, and 0.1 M phosphate, pH 7.40, 37 °C. HMPAL semicarbazone was determined by HPLC at 280 nm. K_i value for the inhibition of HMPE oxidation by ethanol is 23 μM.

problems due to instability of DHPE at higher pH. Comparative data on ethanol oxidation at pH 7.40 have been presented (Mårdh et al., 1985). Except for $\beta_1\beta_1$ ADH, all class I isozymes readily oxidize DHPE and HMPE with k_{cat} values between 2.3 and 12 min^{-1}, overlapping those for ethanol oxidation, i.e., 9.8 and 39 min^{-1} (Table I). Compared to ethanol, the K_m values for DHPE and HMPE are consistently lower. Thus, the k_{cat}/K_m ratios of $\alpha\gamma_1$, $\alpha\gamma_2$, $\beta_1\gamma_1$, and $\beta_1\gamma_2$ ADH are 2–4-fold higher toward HMPE as compared with those for ethanol, similar to $\alpha\gamma_1$ and $\alpha\beta_1$ ADH, which are 4–5-fold higher for DHPE.

The product of HMPE oxidation, identified as (4-hydroxy-3-methoxyphenyl)acetaldehyde (HMPAL) by formation of its semicarbazone adduct, elutes with the same HPLC retention time as authentic HMPAL semicarbazone. Figure 1 shows typical elution profiles, which monitor its formation during catalysis and also depict the effect of 1.0 mM ethanol. As noted, ethanol inhibits HMPE oxidation competitively, K_i = 23 μM (Figure 2).

1,10-Phenanthroline (OP) competitively inhibits the $\beta_1\gamma_1$ ADH catalyzed oxidation of both HMPE and ethanol with K_i values of 20 and 22 μM, respectively, demonstrating their competition for the active site of the enzyme [Figure 3 and Mårdh et al. (1985)].

Table II compares kinetic parameters for the reduction of the corresponding aldehydes, DHPAL and HMPAL, in the presence of enzyme-saturating concentrations of NADH, pH 7.4, 25 °C, with analogous data for acetaldehyde (Deetz et al., 1984). The K_m values of individual isozymes differ less than 2-fold for the two dopamine aldehydes and range from 8.9 to 240 μM, with the exception of $\beta_1\beta_1$ whose K_m values differ by 20-fold. With $\beta_1\gamma_1$ ADH, the K_m values, 8.9 and

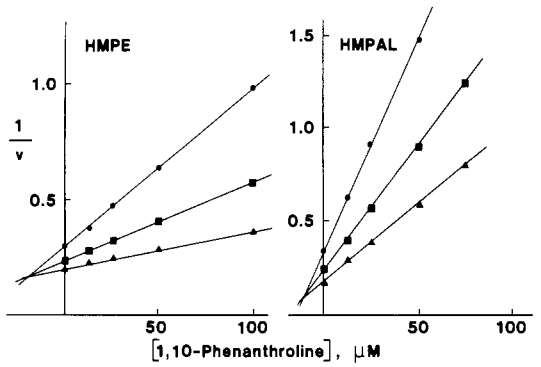

FIGURE 3: Instantaneous inhibition of $\beta_1\gamma_2$ ADH oxidation of HMPE and reduction of HMPAL by OP. HMPE at 100 (●), 200 (■) and 500 (▲) μM and HMPAL at 17.5 (●), 35 (■) and 70 (▲) μM were assayed in mixtures of 2.5 mM NAD^+ or 0.25 mM NADH, 0.20 μM enzyme, and 0.1 M phosphate, pH 7.40, 25 °C. K_i values for inhibition of HMPE oxidation and HMPAL reduction are 20 and 12 μM, respectively. v is expressed as μmol h^{-1} (mg of protein)$^{-1}$.

11 μM, are the lowest for DHPAL and HMPAL, respectively, whereas those with $\beta_1\gamma_2$ ADH are the highest, 240 and 150 μM. All k_{cat} values are between 390 and 4100 min^{-1} except those of $\beta_1\beta_1$ ADH, which are 28 and 4.4 min^{-1}. Consequently, k_{cat}/K_m values generally range from 7800 to 190 000 mM^{-1} min^{-1}, an order of magnitude higher than those for the reduction of acetaldehyde, 780–4900 mM^{-1} min^{-1}. $\beta_1\gamma_1$ and $\beta_1\beta_1$ ADH are the ones that are most strikingly different. For the reduction of HMPAL and DHPAL, the k_{cat}/K_m of $\beta_1\gamma_1$ is 110- and 240-fold greater, respectively, than for that of acetaldehyde, while for $\beta_1\beta_1$ it is 700- and 5000-fold less than that of $\beta_1\gamma_1$. Thus, $\beta_1\gamma_1$ is most effective in reducing HMPAL and DHPAL, while $\beta_1\beta_1$ is nearly as efficient in reducing acetaldehyde.

Incubation mixtures containing each dopamine aldehyde as well as NADH and $\beta_1\gamma_2$ ADH yielded the corresponding alcohols, (3,4-dihydroxyphenyl)ethanol and (4-hydroxy-3-methoxyphenyl)ethanol (homovanillyl alcohol), respectively, as identified by HPLC compared with authentic samples.

1,10-Phenanthroline inhibits HMPAL reduction with a K_i of 12 μM, slightly lower than that obtained for inhibition of HMPE oxidation (Figure 3). The value for ethanol oxidation, 22 μM (Mårdh et al., 1985), is closely similar, indicating that the mechanism for OP inhibition is the same.

Discussion

Class I, II, and III ADH isozymes are now well characterized (Vallee, 1985). They differ significantly in substrate specificities, inhibition characteristics, and immunology. There are both inter- and intraclass differences of their substrate specificities (Wagner et al., 1983, 1984; Ditlow et al., 1984). Methanol, digitoxigenins, and the glycol intermediates in norepinephrine metabolism solely are substrates of class I ADH (Frey et al., 1980; Wagner et al., 1983, 1984; Ditlow et al., 1984), while class II preferentially catalyzes benzyl alcohol/benzaldehyde oxidoreduction (Deetz et al., 1984). Thus far, only long-chain aliphatic alcohols have been found to be good substrates of class III ADH (Wagner et al., 1984). These differences suggest a basis for the existence of human alcohol dehydrogenase isozymes other than ethanol oxidation and acetaldehyde reduction.

We have further described that class I ADH catalyzes the interconversion of alcohol and aldehyde intermediates of dopamine. The oxidation of HMPE and DHPE is analogous to that of the norepinephrine glycols by class I ADH (Mårdh et al., 1985). Though in the human neither HMPE nor DHPE has been shown to be oxidized in vivo, the present results are consistent with observations of a pyrazole-sensitive HMPE oxidation in the rat, suggesting that HMPE and DHPE are oxidized to homovanillic and (3,4-dihydroxyphenyl)acetic acids in vivo.

Ethanol competes very effectively for ADH to decrease the formation of acid metabolites via this route. The similarity of the manner in which the oxidation of HMPE, DHPE, ethanol, and the norepinephrine glycols is inhibited by 1,10-phenanthroline (Figures 3) (Mårdh et al., 1985) points to the fact that all of these compete for one active site of ADH. However, the K_i value for ethanol inhibition of $\beta_1\gamma_2$ ADH catalyzed HMPE oxidation is 23 μM (Figure 2), 10-fold lower than the apparent K_m value for ethanol in the substrate concentration range of from 250 μM to 5.0 mM (Table 1). One explanation for the difference in the K_i and K_m values observed could be that ethanol can recognize a noncatalytic but potentially regulatory site. The mechanism of the ethanol and the dopamine alcohol interaction clearly calls for further elucidation. Interestingly, ethanol and the intermediary alcohol of serotonin also compete for class I ADH isozymes but in that instance with K_i values comparable to their apparent K_m values for ethanol (unpublished manuscripts).

While the k_{cat}/K_m ratios of class I ADH catalyzed interconversion of norepinephrine alcohols and aldehydes are similar both in the oxidative and in the reductive directions [Mårdh et al. (1985) and unpublished observations], class I ADH is extremely effective in reducing the dopamine aldehydes (Table II).

The physiological significance of this observation is not clear, however, due to a number of factors, among them principally the virtual absence of class I ADH in the central nervous system (Beisswenger et al., 1985). In addition, the NAD/NADH ratio for reduction of aldehydes by ADH is unfavorable (Forsander et al., 1958), and further, this reaction would seem competitive with aldehyde reductases. In point of fact, the virtual absence of class I ADH in the brain (Beiswenger et al., 1985) does not preclude the occurrence of ADH reduction of dopamine aldehydes in other tissues that are rich in class I isozymes, e.g., the liver. The present data do not address this question, and further work on tissue specificity of these particular reactions is needed.

The redox state of the tissues, as reflected in NAD/NADH ratios, may exclude reduction by ADH under normal conditions. Yet, during concurrent alcohol oxidation or other reactions that provide NADH, such reductions could take place, e.g., when catalyzed by the enzyme–NADH complex (Gupta & Robinson, 1966).

The third factor that is important for physiological ADH reduction encompasses the aldehyde reductases that have been purified partially from various tissue and species and have been shown capable of reducing the dopamine aldehydes with K_m values similar to those obtained in the present study (Erwin & Deitrich, 1966; Wermuth & Münch, 1979; von Wartburg & Wermuth, 1982). It is as yet unclear whether or not an apparently nonspecific aldehyde reductase (Flynn, 1982) or an alcohol dehydrogenase or both may be involved in dopamine metabolism.

Support for the participation of an alcohol dehydrogenase in dopamine metabolism, however, can be drawn from studies on the effects of concurrent ethanol administration. For one, ethanol shifts dopamine metabolism toward the formation of increased amounts of reduced metabolites, i.e., HMPE and DHPE (Davis et al., 1970). Second, in the rat HMPE is oxidized to homovanillic acid, and this oxidation appears to

be inhibited by pyrazole (Waterbury et al., 1973). HMPE has also been found to be oxidized by horse liver ADH in vitro, a reaction that is inhibited competitively by pyrazole in a fashion similar to that of ethanol (Waterbury et al., 1973). Third, in vitro studies with rat liver have revealed that ADH inhibitors prevent the effects of ethanol on dopamine metabolism (Tank & Weiner, 1979). Importantly, changes in cytosolic NAD/NADH ratios do not alter dopamine metabolism, nor does acetaldehyde promote formation of alcohol metabolites. In addition, 5β-androstan-3β-ol-17-one, an alternative substrate for ADH [Waller et al. (1965) and unpublished work], mimics the effect of ethanol (Tank & Weiner, 1979).

Thus, it appears highly unlikely that changes in NAD/NADH ratios or specificity differences of aldehyde dehydrogenase toward acetaldehyde and the dopamine aldehydes can account for the ethanol-induced alteration of dopamine metabolism. On the other hand, in the light of the present results, it seems that the metabolic changes subsequent to ethanol intake can be explained on the basis of and competition by ethanol/acetaldehyde and the dopamine intermediates for ADH. Further work on the disposition of the alcohol metabolites in man is needed in order to delineate the extent of HMPE and DHPE oxidation relative to that of the formation of the corresponding acid metabolites.

It is not apparent that class I ADH isozymes catalyze the interconversion of dopamine intermediary alcohols and aldehydes, thereby providing a basis for further understanding of ethanol-induced alterations of the metabolic patterns of dopamine. This circumstance may prove important in the interpretation of the interactions between ethanol and neurotransmitters and the consequent metabolic patterns.

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Registry No. ADH, 9031-72-5; DHPE, 10597-60-1; HMPAL, 5703-24-2; HMPE, 2380-78-1; OP, 66-71-7; DHPAL, 5707-55-1; AcH, 75-07-0; EtOH, 64-17-5; dopamine, 51-61-6.

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