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Reduction of biogenic aldehydes by aldehyde reductase and alcohol dehydrogenase from human liver

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Biogenic aldehydes are formed by oxidative deamination of the biogenic amines by monoamine oxidase. In the central nervous system of man [1] and throughout the body of the rat [2, 3] and other species [4, 5] the aldehydes derived from phenylethylamines (e.g., dopamine) and indoleamines (e.g., serotonin) are further oxidized to the corresponding acids by NAD-dependent aldehyde dehydrogenase, whereas the aldehydes derived from β-hydroxylated phenylethylamines (e.g. epinephrine, norepinephrine) are reduced to the glycol derivatives by NADPH-linked aldehyde reductase. However, in the liver and other peripheral tissues of man the aldehydes of epinephrine and norepinephrine are oxidized rather than reduced [6].

Smith *et al.* [7, 8] and Davis *et al.* [9, 10] were the first to demonstrate that ingestion of ethanol elicits a shift in the catabolic pathways of norepinephrine and serotonin, respectively, that favors the formation of reduced as opposed to oxidized metabolites. It soon became evident that this effect of ethanol on the biogenic aldehyde metabolism could not be explained by a single mechanism. At this point, two mechanisms, corroborated by experimental data, have been proposed to account for the observed alterations in the catabolic pathways of the biogenic amines after ingestion of ethanol. One proposal is that the effect of ethanol occurs via its oxidation product, acetaldehyde, which competitively inhibits its aldehyde dehydrogenase [8, 11]. Inhibition of the oxidative pathway causes an increased steady state concentration of the biogenic aldehydes, which alternatively are reduced by aldehyde reductase. This mechanism appears to be effective mainly for norepinephrine. The other mechanism, which was shown to be operative for dopamine [12], is based on the finding that ethanol mediates the reduction of aldehydes by alcohol dehydrogenase via a coupled oxidation–reduction shuttle between the ethanol and the aldehyde [13]. For alcohol dehydrogenase the rate-limiting step in the conversion of ethanol to acetaldehyde is the release of NADH from the enzyme. Thus, in the presence of ethanol, a sizeable steady state concentration of enzyme–NADH complex is formed that may bind and reduce biogenic aldehydes even under conditions of a high cellular NAD^+/NADH ratio.

In order to evaluate the factors that determine the metabolism of the biogenic aldehydes either by aldehyde reductase or by alcohol dehydrogenase, we have investigated the reduction of various biogenic aldehydes by aldehyde reductase and alcohol dehydrogenase.

Materials. Biogenic amines were obtained either from Sigma, St. Louis, USA or from Fluka, Buchs, Switzerland. Indoleacetaldehyde sodium bisulfite was a product of Regis, Morton Grove, USA.

Enzymes. Aldehyde reductase (EC 1.1.1.2) was purified from human liver as described previously [14]. Alcohol dehydrogenase (EC 1.1.1.1) was isolated from the same livers and separated from aldehyde reductase by DEAE-cellulose chromatography. Alcohol dehydrogenase, which does not bind to the cellulose, was fractionated by ammonium sulfate precipitation between 35 and 80 per cent saturation. The precipitate was collected by centrifugation and dialyzed against 0.05 M sodium phosphate, pH 7.4. This preparation, consisting of a mixture of alcohol dehydrogenase isozymes [15], was used for all subsequent determinations.

Mitochondrial outer membranes, isolated from calf liver by the method of Parsons *et al.* [16], were used as source of monoamine oxidase (EC 1.4.3.4).

Assay procedures. Aldehyde reductase and alcohol dehydrogenase were assayed by monitoring the decrease in absorbance of NADPH or NADH, respectively, at 340 nm.

Calculation of kinetic constants. Values of K_m and V_{max} were determined by graphical extrapolation from double reciprocal plots [17]. Six to 10 different concentrations of aldehyde were used for each determination of kinetic constants, and the line of best fit was computed by the least squares method.

Preparation of aldehydes. Free indoleacetaldehyde was prepared from its sodium bisulfite adduct by alkaline hydrolysis. Other aldehydes (listed in the Table) were prepared by incubation of the appropriate amine with monoamine oxidase according to the procedure of Tabakoff *et al.* [2]. The concentration of aldehydes was determined enzymatically: aliquots of the aldehyde solutions (ca. 50 nmol) were incubated with aldehyde reductase and alcohol dehydrogenase, respectively, in 1 ml 0.8 M sodium phosphate buffer, pH 7.0, containing 0.3 mM of the appropriate reduced coenzyme. The concentration of the aldehyde was calculated from the decrease in absorbance at 340 nm.

Both enzymes, aldehyde reductase and alcohol dehydrogenase, reduced all the biogenic aldehydes tested in this study, and in all cases linear graphs were obtained in a double reciprocal representation of kinetic data. Figure 1 depicts the Lineweaver–Burk plots of the aldehyde reductase and alcohol dehydrogenase-catalyzed reduction of 3-methoxy-4-hydroxyphenylglycolaldehyde, the main deamination product of epinephrine and norepinephrine in human peripheral tissues. Table 1 lists the kinetic constants K_m and V_{max} of aldehyde reductase and alcohol dehydrogenase for eight biogenic aldehydes. These data are discussed below in a separate section for each enzyme.

Aldehyde reductase. Glycolaldehydes had 10 to 20-fold lower Michaelis constants and were reduced at a rate 5 to 10 times higher than the corresponding aldehydes bearing no hydroxyl group on the aldehyde-containing side chain. In fact, the aldehydes derived from octopamine and (nor-)epinephrine are among the best substrates of human liver aldehyde reductase. A similar preference for the glycolaldehydes over the acetaldehyde-type derivatives has been reported for brain aldehyde reductase of several species [2, 4, 5], although the differences in the Michaelis constants are less pronounced for the brain than for the liver reductase.

The low K_m values for the glycolaldehyde derivatives suggest that an increase in the steady state concentrations of biogenic aldehydes by inhibition of aldehyde dehydrogenase should primarily affect the catabolism of epinephrine and norepinephrine. Smith and Gitlow have shown that treatment of healthy human subjects with disulfiram, an inhibitor of the cytosolic aldehyde dehydrogenase from human liver [18], indeed shifts the catabolism of norepinephrine towards a reductive pathway, but does not alter the predominantly oxidative catabolism of tryptamine [8].

In our study indoleacetaldehyde and 5-hydroxyindoleacetaldehyde were poor substrates for aldehyde reductase. This finding differs from results obtained with an aldehyde reductase from rat liver [19], which reduces indoleacetaldehyde about ten times as fast as 4-hydroxyphenylglycoaldehyde. However, the K_m and V_{max} values for the two aldehydes have not been determined with the rat liver enzyme.

Table 1. Michaelis constants and relative maximal velocities for biogenic aldehydes of aldehyde reductase and alcohol dehydrogenase from human liver

Aldehyde	Aldehyde reductase		Alcohol dehydrogenase	
	K_m (μM)	V_{max} (%)	K_m (μM)	V_{max} (%)
4-Hydroxyphenylacetaldehyde	1030, 1190	27, 58	10, 19	130, 150
4-Hydroxyphenylglycolaldehyde	50, 63	106, 138	56, 63	29
3,4-Dihydroxyphenylacetaldehyde	460, 627	13, 15	15, 19	56, 94
3,4-Dihydroxyphenylglycolaldehyde	35, 56	112, 129	18, 20	9, 18
3-Methoxy-4-hydroxyphenylacetaldehyde	882	10	8, 12	72, 93
3-Methoxy-4-hydroxyphenylglycolaldehyde	41, 45	49, 69	11, 34	13, 23
Indoleacetaldehyde	302, 527	11, 15	6,10	180, 198
5-Hydroxyindoleacetaldehyde	227	9	3, 6	38, 50

Kinetic constants were extrapolated from graphic representations of data by the method of Lineweaver and Burk [17]. Velocities are expressed as per cent of the activity obtained with the same amount of enzyme in the presence of 0.5 mM 4-nitrobenzaldehyde and 0.08 mM reduced coenzyme. Multiple values are results obtained with different aldehyde preparations.

Based on the reduction of 4-nitrobenzaldehyde the amount of aldehyde reductase activity is one order of magnitude higher in the liver than in the brain [14, 20, see also legend to Fig. 1], yet *in vivo* biogenic aldehydes are not reduced to an appreciable degree in the liver as they are in the brain.

Kraemer and Deitrich have shown that human liver aldehyde dehydrogenase is localized mainly in the cytosolic compartment of the cell [18]. On the other hand, in the central nervous system of mammals aldehyde dehydrogenase is present predominantly in the mitochondria [21]. Thus, in the liver aldehyde dehydrogenase and aldehyde reductase may compete more effectively for the various biogenic aldehydes than they do in the brain.

Anderson *et al.* [22] have suggested that in the central nervous system biogenic aldehydes are metabolized by a mitochondrial rather than by the cytosolic aldehyde reductase corresponding to the liver enzyme, and the absence of such an isozyme in the liver could account for the observed differences in the metabolic pattern of biogenic amines.

Alcohol dehydrogenase. The presence of a hydroxyl group on the side chain decreased the rate of reduction of aldehydes to about one-fifth of the value measured with the corresponding aldehydes bearing no hydroxyl group on the side chain. A similar difference was observed between the rates of reduction of indoleacetaldehyde and its 5-hydroxyl derivative. The presence of hydroxyl groups, either on the side chain or in the ring system, however, little influenced the Michaelis constants, which are of the same order of magnitude as those for aliphatic aldehyde substrates [23]. Duncan has reported K_m values for the aldehydes of serotonin and dopamine with alcohol dehydrogenase from horse liver which are 5 to 10 times higher than those obtained in this study with the human enzyme [24]. The aldehyde derived from norepinephrine was no substrate for the horse liver enzyme, a finding that may be explained by the narrower substrate specificity of horse liver alcohol dehydrogenase as compared to that from human liver.

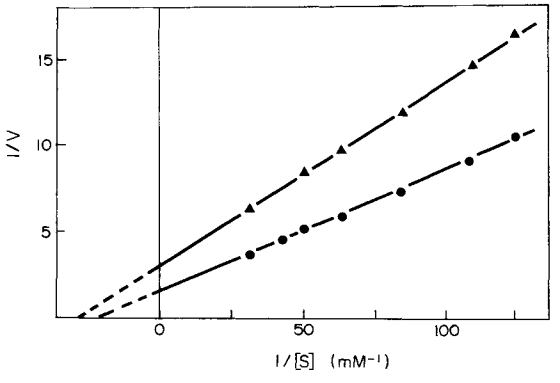

Fig. 1. Lineweaver–Burk plot of the reduction of 3-methoxy-4-hydroxyphenylglycolaldehyde by aldehyde reductase and alcohol dehydrogenase from human liver.

The enzymes were assayed as indicated in the experimental section. The reaction mixture contained in a total vol. of 1 ml: 0.08 M sodium phosphate, pH 7.0, 0.08 mM reduced coenzyme and variable amounts of aldehyde. The reaction was started by the addition of the enzyme. The symbols (●) for aldehyde reductase and (▲) for alcohol dehydrogenase represent mean values of at least two determinations.

Based on the reduction of 4-nitrobenzaldehyde, in human liver the amount of alcohol dehydrogenase activity is about 40 times as high as the amount of aldehyde reductase activity. In the absence of ethanol alcohol dehydrogenase, however, may be prevented *in vivo* from acting as a reductase by the high cellular ratio of $NAD^+/NADH$.

Several authors have suggested that ingestion of ethanol and a subsequent inhibition of aldehyde dehydrogenase by acetaldehyde will cause an increase in the concentration of the biogenic aldehydes leading to a spontaneous formation of pharmacologically active condensation products between the aldehydes and the parent amines [25, 26]. The Michaelis constants obtained in this study with alcohol dehydrogenase indicate that, in the presence of ethanol, the steady state concentrations of the various aldehydes will not exceed a few micromoles per liter.

Biogenic aldehydes are reduced *in vitro* by aldehyde reductase and by alcohol dehydrogenase from human liver. Aldehyde reductase preferentially reduces the aldehydes derived from β-hydroxylated amines, whereas the aldehydes bearing no hydroxyl group on the carbonyl-containing side chain are better substrates for alcohol dehydrogenase. These differences help to explain the diverging effects of aldehyde dehydrogenase inhibitors on the peripheral catabolism of norepinephrine and serotonin, respectively in the presence and in the absence of ethanol.

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Distribution of loperamide in the intestinal wall

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Loperamide [4-(4-chorophenyl)-4-hydroxy-*N*, *N*-dimethyl-α,α-diphenyl-1-piperidinebutanamide monohydrochloride], is a potent orally active antidiarrheal compound without central narcotic properties [1–3]. In the guinea pig ileum it selectively represses peristalsis by inhibiting the local reflex control of intestinal motility [4]. Loperamide binds to membrane sites in the longitudinal muscle–myenteric plexus preparation of the guinea pig ileum [5–7]. Studies of the distribution of labelled loperamide in rats indicate that a large part of the drug is found in the digestive tract [8, 9].

The aim of the present study was to determine, by radioimmunoassay, how exogenously added loperamide is distributed throughout the wall of the isolated guinea pig ileum.

Guinea pigs (350 to 400 g; $n = 19$) were killed by cervical translocation. The non-terminal ileum was isolated, and incubated in 100 ml Tyrode solution kept at 37° and aerated with a gas mixture of 95% O_2–5% CO_2 (pH 7.4). The incubation solution contained loperamide at concentrations of 1.25, 5 or 20 ng/ml; these concentrations produce an average of 7, 44 and 100 per cent inhibition of the peristaltic reflex activity, respectively [4]. At the end of the incubation period the ileum was washed repeatedly with drug-free Tyrode solutions. Six tissues were then prepared for radioimmunological determination of loperamide: an intact segment, a segment without mucosa, the longitudinal muscle with the myenteric plexus attached to it, the circular muscle with the mucosa, the circular muscle without the mucosa and the mucosa. Five to eight experiments were done per concentration of loperamide.

To determine the concentration of loperamide, different parts of the ileum, blotted with filter paper, were weighed and homogenized in methanol (1:5 w:v). After centrifugation, the supernatant was evaporated to dryness. The residue was dissolved in 0.05 ml of methanol and the concentration of loperamide was determined by radioimmunoassay as previously described [10]. Briefly, 0.2 ml of a 1/130 antiserum dilution and 0.5 ng of tritium-labelled loperamide (sp. act. 9 Ci/mM) was added to 0.05 ml of the reconstituted tissue extracts. The mixture was diluted with phosphate buffer (0.05 M, pH 7.4) to a final volume of 0.8 ml and incubated for 2 hr at room temperature. After the incubation period, free and specifically bound loperamide were separated by the addition of a suspension of dextran-coated charcoal (0.2 ml of a 0.5% suspension). The mixture was allowed to equilibrate for 1 hr at room temperature. The charcoal was then removed by centrifugation, and the radioactivity present in the supernatant was determined by liquid scintillation counting (Packard Tri-Carb, Model 3380, with an absolute activity analyzer model 544).

The uptake of loperamide by the 6 types of preparation is shown in Table 1. Loperamide accumulated in the longitudinal muscle–myenteric plexus preparation. The selective uptake of loperamide by the longitudinal muscle–myenteric plexus preparation was most pronounced at those concentrations inhibiting peristaltic reflex activity [4]. At these concentrations of 5 and 20 ng/ml the amounts of drug in the longitudinal muscle–myenteric plexus preparation, expressed per gram tissue, were respectively 9 and 9.5 times higher than in the whole ileal segment, and 8 to 12 times higher than in the circular muscle. The small amounts found in the mucosa were negligible, being near the limit of detection (±0.60 ng/g).

Both the selective uptake by the longitudinal muscle–myenteric plexus preparation and the inhibition of the peristaltic reflex activity increased with increasing loperamide concentrations in the incubation medium. This implies that the loperamide-induced inhibition of intestinal motility depends upon the amount of active drug reaching the longitudi-